

FLUORINATED PHOTSENSITIZERS RELATED TO CHLORINS AND BACTERIOCHLORINS FOR PHOTODYNAMIC THERAPY

RELATED APPLICATIONS

Benefit of priority under 35 U.S.C. § 119(e) to the following provisional
5 application is claimed herein: U.S. provisional application 60/392,473 to
Pandey *et al.*, filed June 27, 2002, entitled "FLUORINATED
PHOTSENSITIZERS RELATED TO CHLORINS AND
BACTERIOCHLORINS FOR PHOTODYNAMIC THERAPY."

The above-noted provisional application is incorporated by reference
10 herein in its entirety.

FIELD

Provided herein are compounds for detection, diagnosis and treatment
of target tissues or target compositions, including hyperproliferative tissues
such as tumors, using photodynamic methods. In particular, photosensitizer
15 compounds that collect in hyperproliferative tissue are provided. In another
embodiment, compounds that absorb light at a wavelength of from about 700
to about 850 nm are provided. In a further embodiment, compounds that are
detectable by magnetic resonance imaging are provided.

BACKGROUND

20 Photodynamic therapy ("PDT") is a process whereby light of a specific
wavelength is directed to tissues undergoing treatment or investigation that
have been rendered photosensitive through the administration of a
photoreactive or photosensitizing agent. The objective of the intervention may
be either diagnostic, where the wavelength of light is selected to cause the
25 photoreactive agent to fluoresce, thus yielding information about the tissue
without damaging the tissue, or therapeutic, where the wavelength of light
delivered to the photosensitive tissue under treatment causes the photo-
reactive agent to undergo a photochemical interaction with oxygen in the
tissue under treatment that yields high energy species, such as singlet oxygen,
30 causing local tissue lysing or destruction. The method of van Lier

(*Photobiological Techniques 216*: 85-98 (Valenzo *et al.* eds. 1991)) can be used to confirm the ability of any given compound to generate singlet oxygen effectively, thus making it a good candidate for use in photodynamic therapy.

In photodynamic therapy, a photosensitizer compound that

5 demonstrates the ability to selectively accumulate in target tissue, such as neoplastic or hyperproliferative tissue, is administered to a subject, and when the photosensitizer accumulates in or preferentially associates with the target tissue, the target tissue becomes sensitized to photoradiation. The photo-

10 sensitizing agent can be activated either coherent (laser) or non-coherent (non-laser) light. It is currently accepted that following absorption of light, the photosensitizer is transformed from its ground singlet state (P) into an electronically excited triplet state ($^3P^*$; $\tau \sim 10^{-2}$ sec.) via a short-lived excited singlet state ($^1P^*$; $\tau \sim 10^{-6}$ sec.) The excited triplet can undergo non-radiative decay or participate in an electron transfer process with biological substrates

15 to form radicals and radical ions, which can produce singlet oxygen and superoxide (O_2^-) after interaction with molecular oxygen (O_2). Singlet oxygen can be produced from molecular oxygen by the transfer of energy directly or indirectly from the activated photosensitizer. Singlet oxygen is one of the agents responsible for cellular and tissue damage in PDT, causing oxidation of

20 the target tissue; there also is evidence that the superoxide ion may be involved. The generation of these cytotoxic agents plays a role in tumor homeostasis and the observed tumor destruction.

Photodynamic therapy has proven to be very effective in destroying abnormal tissue such as cancer cells. In this therapy, a photoreactive agent

25 having a characteristic light absorption wavelength or waveband is first administered to the patient, typically either orally or by injection. Abnormal tissue in the body is known to selectively absorb certain photoreactive agents to a much greater extent than normal tissue, e.g., tumors of the pancreas and colon may absorb two to three times the volume of these agents, compared to

30 normal tissue. Certain porphyrins and related tetrapyrrolic compounds tend to localize in abnormal tissue, including malignant tumors and other hyperproliferative tissue, such as hyperproliferative blood vessels, at much

higher concentrations than in normal tissues, so they are useful as a tool for the treatment of various type of cancers and other hyperproliferative tissue by photodynamic therapy (PDT) (T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Kprbelik, J. Moan, Q. Peng, *J. Natl. Cancer Inst.* 90: 889
5 (1998), incorporated here by reference). However, most of the porphyrin-based photosensitizers including PHOTOFRIN[®] (a purified hematoporphyrin derivative (HpD) approved worldwide for the treatment of tumors) clear slowly from normal tissue, so patients must avoid exposure to sunlight for a significant time after treatment.

10 PDT with PHOTOFRIN[®] has been used to treat a multiplicity of tumors accessible to light, including skin, lung, bladder, head and neck, breast, gastric, cervical and esophageal cancers. PHOTOFRIN[®] has some desirable characteristics, including good efficacy, water solubility, good yield of singlet oxygen, and ease of manufacture. However, PHOTOFRIN[®] has some
15 disadvantageous properties: (i) it is a complex mixture of porphyrin dimers and higher oligomers linked by ether, ester, and/or carbon-carbon bonds and, therefore is difficult to study; (ii) it shows skin phototoxicity in patients for four to six weeks after administration; and (iii) due to its relatively weak absorbance in the red region (630 nm), lack of penetration of light through tissue limits
20 current clinical applications of PHOTOFRIN[®] in PDT to the destruction of cancerous tissue less than 4 mm from the source of light used in the therapy.

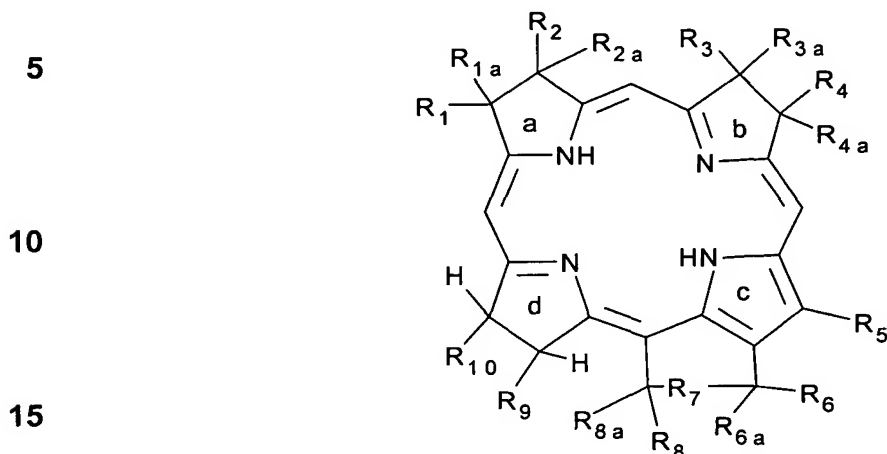
Thus, there is a need for additional photosensitizers for use in PDT, diagnostic and therapeutic applications.

SUMMARY

25 Provided herein are fluorinated compounds for use in PDT, diagnostic and therapeutic applications. In one embodiment, the compounds preferentially absorb into target tissue, including hyperproliferative tissue. In another embodiment, the compounds absorb light at a wavelength of between about 700 and about 850 nm.

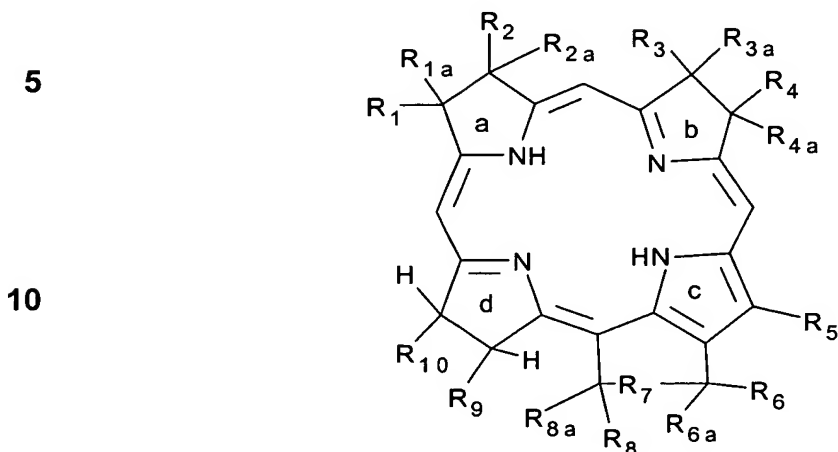
30 In one embodiment, provided herein are tetrapyrrole compounds containing a fluorinated substituent where the compound is a chlorin or bacteriochlorin.

Also provided herein are compounds of the formula:



or a pharmaceutically acceptable derivative thereof, where R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_5 , R_6 , R_{6a} , R_8 , R_{8a} , R_9 , and R_{10} are independently hydrogen, lower alkyl of about 1 through 8 carbon atoms, lower alkenyl of about 1 through 8 carbon atoms, or lower alkyl of about 1 through 8 carbon atoms substituted with at least one halogen, hydroxy, carboxy, ester, aromatic, heterocyclic, ether, amide, or amine group; where two R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{4a} , R_6 , R_{6a} , R_8 , R_{8a} , R_9 and R_{10} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_6 , R_{6a} , R_8 , and R_{8a} groups on the same carbon atom may form a double bond to a divalent pendant group; R_1 or R_2 may additionally be $-\text{CH}=\text{CH}_2$, $-\text{CHO}$, $-\text{COOH}$, $-\text{COOR}_a$, or $\text{H}_3\text{C}-\text{CH}(\text{OR}_{11})$; R_7 is $-\text{CH}_2-$, or $=\text{NR}_{12}$, or a covalent bond, where R_{11} and R_{12} are independently hydrogen, lower alkyl of about 1 through 8 carbon atoms, lower alkenyl of about 1 through 8 carbon atoms, or lower alkyl of about 1 through 8 carbon atoms substituted with at least one halogen, hydroxy, carboxy, ester, aromatic, heterocyclic, ether, amino acid, amide, or amine group; provided that at least one of R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_5 , R_6 , R_{6a} , R_7 , R_8 , R_{8a} , R_9 and R_{10} contains at least one fluorinated pendant group selected from the group consisting of fluorinated alkyl groups, fluorinated phenyl groups and fluorinated heterocyclic moieties.

Also provided are compounds of the formula



or a pharmaceutically acceptable derivative thereof, where R_1 and R_2 are each

15 independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, $-C(O)R_a$ or $-COOR_a$, where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or

20 together form a covalent bond; R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl; R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond; R_5 is hydrogen or substituted or unsubstituted alkyl; R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form

25 $=O$; R_7 is a covalent bond, alkylene, azaalkyl, or azaaralkyl; R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form $=O$; R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl; each of R_1 - R_{10} , when substituted, is substituted with one or more substituents, in one embodiment one to five substituents, in another

30 embodiment one, two or three substituents, each independently selected from Q , where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl,

- aralkyl, or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=\text{NR}_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue; each Q is independently unsubstituted or is substituted with one or more substituents, in one embodiment one to five substituents, in another embodiment one, two or three substituents, each independently selected from Q_1 , where Q_1 is alkyl, haloalkyl, halo, pseudohalo, $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue.
- 15 In another embodiment the compounds are selected with the proviso that the compound contains at least one fluorine atom.
- Also provided are methods for detecting target tissue or target compositions. Further provided herein is a method for photodynamic therapy using the compounds provided herein. Also provided herein is a method for
- 20 detecting hyperproliferative tissue using the compounds provided herein.
- Also provided is the use of the compounds provided herein for the treatment of target compositions or target tissue, including hyperproliferative tissue and neovascular tissue. Provided herein is also a method for detecting the presence of hyperproliferative tissue in a subject. Also provided is a
- 25 method of diagnosing hyperproliferative disorders in a patient. Further provided is a method of diagnosing an infecting agent in a patient.
- Provided herein is also a method of generating an image of a target tissue in a subject. Also provided herein is a method of labeling a target tissue for diagnostic radiology. Further provided is a kit to treat hyperproliferative
- 30 disorders. Also provided is a kit to label specific tissues for diagnostic radiology. Further provided is a combination, including a compound provided herein and a light source. Further provided is a combination including a

photosensitizer compound provided herein and a magnetic resonance imaging device.

Brief Description of the Drawings

FIGURE 1 illustrates the electronic absorption spectra of two chlorin based photosensitizers, with that of an existing product (PHOTOFRIN®) shown for comparison.

FIGURE 2 illustrates *in vitro* photosensitizing efficacy of the purpurinimide (chlorin) photosensitizer **9** in RIF tumor cells.

FIGURE 3 illustrates comparative *in vitro* photosensitizing efficacy of four chlorin-based photosensitizers, consisting of a pair of fluorinated compounds (**9**, **12**) and a pair of corresponding non-fluorinated compounds (**8**, **11**).

FIGURE 4 illustrates the *in vivo* photosensitizing efficacy of the fluorinated (**9**, **12**) and corresponding non-fluorinated (**8**, **11**) chlorin photosensitizers of FIGURE 3.

FIGURES 5A and 5B through 8A and 8B illustrate the selective tissue distribution of newly synthesized purpurinimide analogs **8**, **9**, **11** and **12**, respectively, as addressed above in FIGURES 3 and 4.

FIGURE 9 and FIGURE 10 illustrate the *in vivo* photosensitizing efficacy of bacteriochlorins **16** and **18**, respectively, at varying concentrations.

FIGURES 11A and 11B and FIGURES 12A and 12B illustrate the selective tissue distribution of newly synthesized bacteriochlorins **16** and **18**, respectively, addressed above in FIGURES 9 and 10.

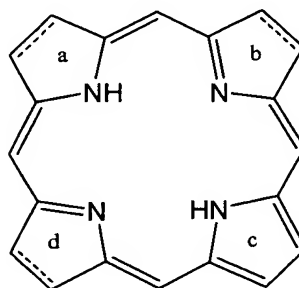
DETAILED DESCRIPTION

A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications are incorporated by reference in their entirety. In the event that there are a

plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

- As used herein, the term "tetrapyrrole compound" denotes a macrocyclic compound containing four pyrrole rings, having the general structure:



where the dashed line indicates that the indicated bond may be saturated or unsaturated, and where any atom of the ring may be substituted or unsubstituted.

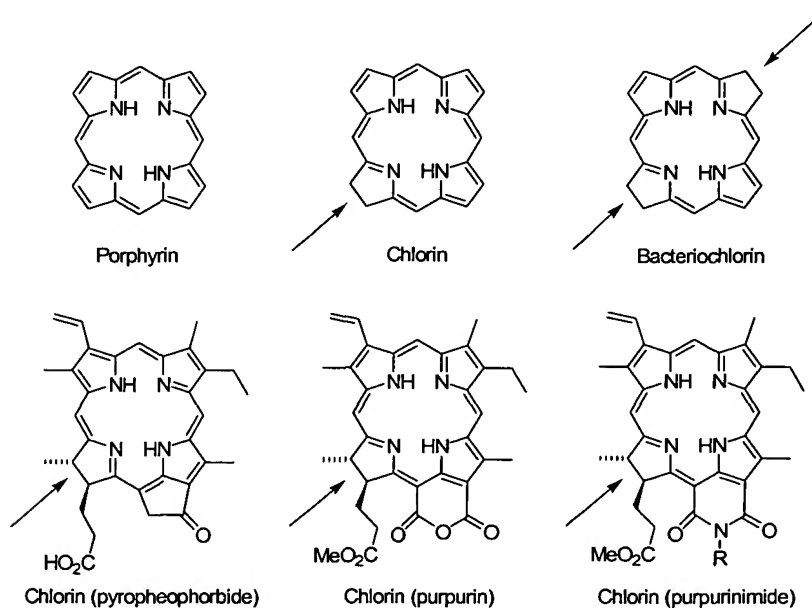
- As used herein, the term "porphyrin" refers to a cyclic structure typically composed of four pyrrole rings, and refers to a porphyrin or porphyrin derivative. Such derivatives include porphyrins with extra rings ortho-fused, or ortho-perifused, to the porphyrin nucleus, porphyrins having a replacement of one or more carbon atoms of the porphyrin ring by an atom of another element (skeletal replacement), derivatives having a replacement of a nitrogen atom of the porphyrin ring by an atom of another element (skeletal replacement of nitrogen), derivatives having substituents other than hydrogen located at the peripheral (meso-, β -) or core atoms of the porphyrin, derivatives with saturation of one or more bonds of the porphyrin (hydroporphyrins, e.g., chlorins, bacteriochlorins, isobacteriochlorins, decahydroporphyrins, corphins, pyrrocorphins, etc.), derivatives obtained by coordination of one or more metals to one or more porphyrin atoms (metalloporphyrins), derivatives having one or more atoms, including pyrrolic and pyrromethenyl units, inserted in the porphyrin ring (expanded porphyrins), derivatives having one or more groups removed from the porphyrin ring (contracted porphyrins, e.g., corrin, corrole) and combinations of the foregoing derivatives (e.g. phthalocyanines,

porphyrazines, naphthalocyanines, subphthalocyanines, and porphyrin isomers).

As used herein, "chlorin" refers to a class of porphyrin derivatives having a cyclic structure typically composed of four pyrrole rings having one partially saturated pyrrole ring, such as the basic chromophore of chlorophyll.

As used herein, "bacteriochlorin" refers to a class of porphyrin derivatives having a cyclic structure typically composed of four pyrrole rings having two partially saturated non-adjacent (*i.e.*, *trans*) pyrrole rings, and "isobacteriochlorin" includes those porphyrin derivatives having a cyclic structure typically composed of four pyrrole rings having two partially saturated adjacent (*i.e.*, *cis*) pyrrole rings.

The principal oxidation states of various tetrapyrroles including porphyrin, chlorin, bacteriochlorin, pyropheophorbide, purpurin and purpurinimide are illustrated below.



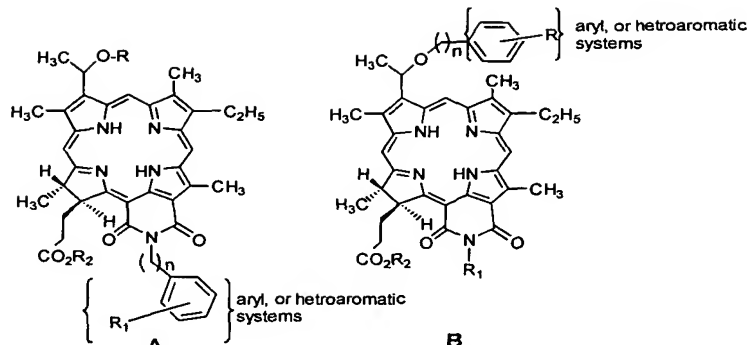
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Various chlorins (structures A and B) and bacteriochlorins (structures C, D and E), are shown below, and exemplify the types of chlorins and bacteriochlorins provided herein.

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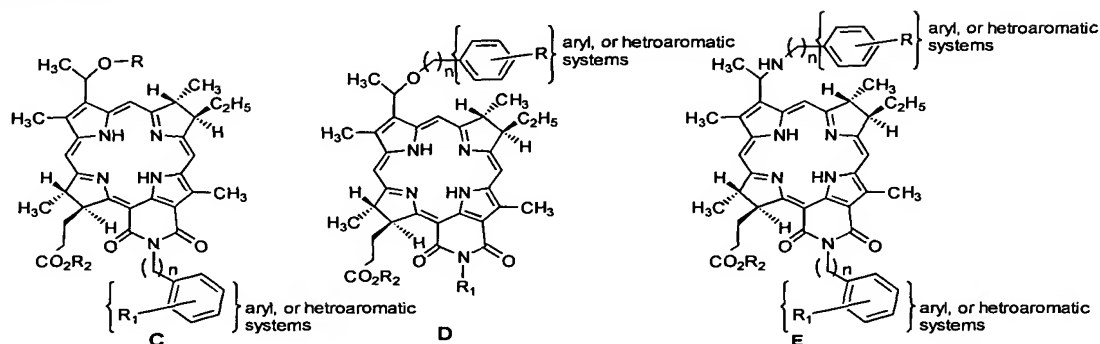
Chlorins:

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Bacteriochlorins

10



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Where R and R_1 = Various alkyl with variable no. of carbon chains their fluorinated analogs, aromatic side chain with fluorinated or non-fluorinated substituents.

R_2 = Fluorinated or non-fluorinated ester groups with variable no. of carbon units, Fluorinated and non-fluorinated amide substituents

As used herein, the recitation "fluorinated" and "fluorinated substituent" denotes replacing at least one atom of a molecule, a group or a substituent with at least one fluorine atom.

As used herein, the term "fluorine atom" denotes all naturally occurring isotopes of fluorine, including, for example, ^{19}F .

As used herein, the term "heterocyclic group" generally refers to a monocyclic or multicyclic saturated, unsaturated, or aromatic carbocyclic group, in one embodiment of from 3 to 20 atoms, including at least one, and preferably a plurality of hetero atoms within the ring, wherein a hetero atom is an element other than carbon, (for example, including but not limited to N, O, S, Se, Te), which hetero atoms may be the same or different. The group may include five- or six-membered heterocyclic rings, polycyclic ring systems, optionally substituted aromatic ring systems having one or more heteroatoms and polyheteroaromatic ring systems where the ring system has from two to four, more preferably two to three, and most preferably two, rings having one

or more hetero atom. The ring systems of the heterocyclic groups may be composed of one ring or two or more rings which may be joined together in a fused, bridged or spiro-connected fashion. In embodiments where the heteroatom(s) is(are) nitrogen, the nitrogen is optionally substituted with alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclalkyl, acyl, guanidino, or the nitrogen may be quaternized to form an ammonium group where the substituents are selected as above. The heteroatom within the ring can optionally be unsubstituted or substituted with, e.g. halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

As used herein, alkyl, alkenyl and alkynyl carbon chains, if not specified, contain from 1 to 20 carbons, or 1 or 2 to 16 carbons, and are straight or branched. Alkenyl carbon chains of from 2 to 20 carbons, in certain embodiments, contain 1 to 8 double bonds and alkenyl carbon chains of 2 to 16 carbons, in certain embodiments, contain 1 to 5 double bonds. Alkynyl carbon chains of from 2 to 20 carbons, in certain embodiments, contain 1 to 8 triple bonds, and the alkynyl carbon chains of 2 to 16 carbons, in certain embodiments, contain 1 to 5 triple bonds. Exemplary alkyl, alkenyl and alkynyl groups herein include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, sec-butyl, tert-butyl, isopentyl, neopentyl, tert-pentyl, isohexyl, allyl (propenyl) and propargyl (propynyl). As used herein, lower alkyl, lower alkenyl, and lower alkynyl refer to carbon chains having from about 1 or about 2 carbons up to about 8 carbons.

As used herein, halogen refers to one of the electronegative elements of group VIIA of the periodic table (fluorine, chlorine, bromine, iodine, astatine).

As used herein, "hydroxy group" generally refers to a hydroxyl group having the formula -OH.

As used herein, "carboxy" generally refers to a divalent radical, -C(O)O-.

As used herein, "ester group" generally refers to a substituent of the general formula $-C-O-O-R^1$ where R^1 may be either aliphatic or aromatic.

As used herein, "aromatic group" generally refers to a ring structure having cyclic clouds of delocalized π electrons above and below the plane of the molecule, where the π clouds contain $(4n+2)$ π electrons. A further discussion of aromaticity is found in Morrison and Boyd, *Organic Chemistry*, (5th Ed., 1987), Chapter 13, entitled "Aromaticity," pages 477-497, incorporated herein by reference.

As used herein, "ether group" generally refers to a compound in which an oxygen atom is bonded to two alkyl or two aryl groups, or one alkyl and one aryl group.

As used herein, "amide group" generally refers to the group $-C(O)NRR$ where each R is independently aliphatic or aromatic.

As used herein, "amine group" has the general formula $-NRR$, where each R is independently any alkyl or aryl group.

As used herein, a fluorinated pendant group generally refers to any substituent that includes at least one fluorine atom.

As used herein, pharmaceutically acceptable derivatives of a compound include salts, esters, enol ethers, enol esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs thereof. Such derivatives may be readily prepared by those of skill in this art using known methods for such derivatization. The compounds produced may be administered to animals or humans without substantial toxic effects and either are pharmaceutically active or are prodrugs.

Pharmaceutically acceptable salts include, but are not limited to, amine salts, such as but not limited to N,N'-dibenzylethylenediamine, chloroprocaine, choline, ammonia, diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethyl-benzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium;

transition metal salts, such as but not limited to zinc; and other metal salts, such as but not limited to sodium hydrogen phosphate and disodium phosphate; and also including, but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates and fumarates. Pharmaceutically acceptable esters include, but are not limited to, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl and heterocyclyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids.

Pharmaceutically acceptable enol ethers include, but are not limited to, derivatives of formula $C=C(OR)$ where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl or heterocyclyl.

Pharmaceutically acceptable enol esters include, but are not limited to, derivatives of formula $C=C(OC(O)R)$ where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl or heterocyclyl.

Pharmaceutically acceptable solvates and hydrates are complexes of a compound with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2, 3 or 4, solvent or water molecules.

As used herein, "cycloalkyl" refers to a saturated mono- or multi- cyclic ring system, in certain embodiments of 3 to 20 carbon atoms, in other embodiments of 3 to 10 carbon atoms; cycloalkenyl and cycloalkynyl refer to mono- or multicyclic ring systems that respectively include at least one double bond and at least one triple bond. Cycloalkenyl and cycloalkynyl groups may, in certain embodiments, contain 3 to 10 carbon atoms, with cycloalkenyl groups, in further embodiments, containing 4 to 7 carbon atoms and cycloalkynyl groups, in further embodiments, containing 8 to 10 carbon atoms. The ring systems of the cycloalkyl, cycloalkenyl and cycloalkynyl groups may be composed of one ring or two or more rings which may be joined together in a fused, bridged or spiro-connected fashion.

As used herein, "aryl" refers to aromatic monocyclic or multicyclic groups containing from 6 to 19 carbon atoms. Aryl groups include, but are not limited to groups such as unsubstituted or substituted fluorenyl, unsubstituted or substituted phenyl, and unsubstituted or substituted naphthyl.

5 As used herein, "heteroaryl" and "heteroaromatic group" refers to a monocyclic or multicyclic aromatic ring system, in certain embodiments, of about 5 to about 20 members where one or more, in one embodiment 1 to 3, of the atoms in the ring system is a heteroatom, that is, an element other than carbon, including but not limited to, nitrogen, oxygen or sulfur. The heteroaryl
10 group may be optionally fused to a benzene ring. Heteroaryl groups include, but are not limited to, furyl, imidazolyl, pyrimidinyl, tetrazolyl, thienyl, pyridyl, pyrrolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, quinolinyl and isoquinolinyl.

As used herein, a "heteroarylium" group is a heteroaryl group that is
15 positively charged on one or more of the heteroatoms.

As used herein, "heterocyclyl" refers to a monocyclic or multicyclic non-aromatic ring system, in one embodiment of 3 to 20 members, in another embodiment of 4 to 10 members, in a further embodiment of 5 to 6 members, where one or more, in certain embodiments, 1 to 3, of the atoms in the ring
20 system is a heteroatom, that is, an element other than carbon, including but not limited to, nitrogen, oxygen or sulfur. In embodiments where the heteroatom(s) is(are) nitrogen, the nitrogen is optionally substituted with alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, acyl, guanidino, or the nitrogen may be
25 quaternized to form an ammonium group where the substituents are selected as above.

As used herein, "aralkyl" refers to an alkyl group in which one of the hydrogen atoms of the alkyl is replaced by an aryl group.

As used herein, "heteroaralkyl" refers to an alkyl group in which one of
30 the hydrogen atoms of the alkyl is replaced by a heteroaryl group.

As used herein, "halo", "halogen" or "halide" refers to F, Cl, Br or I.

As used herein, pseudohalides or pseudohalo groups are groups that behave substantially similar to halides. Such compounds can be used in the same manner and treated in the same manner as halides. Pseudohalides include, but are not limited to, cyanide, cyanate, thiocyanate, selenocyanate, 5 trifluoromethoxy, and azide.

As used herein, "haloalkyl" refers to an alkyl group in which one or more of the hydrogen atoms are replaced by halogen. Such groups include, but are not limited to, chloromethyl, trifluoromethyl and 1-chloro-2-fluoroethyl.

As used herein, "carboxy" refers to a divalent radical, $-C(O)O-$.

10 As used herein, "alkylene" refers to a straight, branched or cyclic, in certain embodiments straight or branched, divalent aliphatic hydrocarbon group, in one embodiment having from 1 to about 20 carbon atoms, in another embodiment having from 1 to 12 carbons. In a further embodiment alkylene includes lower alkylene. There may be optionally inserted along the alkylene 15 group one or more oxygen, sulfur, including $S(=O)$ and $S(=O)_2$ groups, or substituted or unsubstituted nitrogen atoms, including $-NR-$ and $-N^+RR-$ groups, where the nitrogen substituent(s) is(are) alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl or COR' , where R' is alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, $-OY$ or $-NYY$, where Y is hydrogen, alkyl, aryl, heteroaryl, cycloalkyl or 20 heterocyclyl. Alkylene groups include, but are not limited to, methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), propylene ($-(CH_2)_3-$), methylenedioxy ($-O-CH_2-O-$) and ethylenedioxy ($-O-(CH_2)_2-O-$). The term "lower alkylene" refers to alkylene groups having 1 to 6 carbons. In certain embodiments, alkylene groups are lower alkylene, including alkylene of 1 to 3 carbon atoms.

25 As used herein, "substituted alkyl," "substituted alkenyl," "substituted alkynyl," "substituted cycloalkyl," "substituted cycloalkenyl," "substituted cycloalkynyl," "substituted aryl," "substituted heteroaryl," "substituted heterocyclyl," "substituted alkylene," "substituted alkenylene," "substituted alkynylene," "substituted cycloalkylene," "substituted cycloalkenylene," 30 "substituted cycloalkynylene," "substituted arylene," "substituted heteroarylene" and "substituted heterocyclylene" refer to alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heterocyclyl, alkylene,

alkenylene, alkynylene, cycloalkylene, cycloalkenylene, cycloalkynylene, arylene, heteroarylene and heterocyclylene groups, respectively, that are substituted with one or more substituents, in certain embodiments one, two, three or four substituents, where the substituents are as defined herein, in one
 5 embodiment selected from Q¹.

Where the number of any given substituent is not specified (e.g., haloalkyl), there may be one or more substituents present. For example, "haloalkyl" may include one or more of the same or different halogens. As another example, "C₁₋₃alkoxyphenyl" may include one or more of the same or
 10 different alkoxy groups containing one, two or three carbons.

As used herein, "amino acid residue" generally refers to an organic acid carrying an amino group, and are of a general formula ⁺H₃N-CHR-COO⁻. As used herein, "amino acid" refers to natural or unnatural amino acids. The amino acids include but are not limited to 4-aminobutyric acid, 6-amino-
 15 hexanoic acid, alanine, asparagine, aspartic acid, arginine, 3-cyclohexyl-alanine, citrulline, cysteine, 2,4-diaminobutyric acid, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, 3-(2-naphthyl)-alanine, norleucine, ornithine, phenylalanine, 4-halogeno-phenylalanine, phenylglycine, proline, 3-(2-pyridyl)-alanine, serine, 3-(2-thienyl)-alanine,
 20 threonine, tryptophan, tyrosine and valine.

If the amino acids mentioned above can occur in several enantiomeric forms, such as the L- or D-form, then all of these forms and also their mixtures (e.g., the DL-forms) are included as constituents of the compounds disclosed herein. The configuration for naturally occurring amino acid residues is
 25 generally L. When not specified the residue is the L form. The amino acids, e.g., as constituents of compounds disclosed herein, may furthermore be provided with appropriate protective groups known to those skilled in the art. Preferred protecting groups are, for example, BOC (tert-butoxycarbonyl) and Fmoc (9-fluorenylmethoxy-carbonyl) for the N-terminus and OMe (methyl
 30 ester) and OEt (ethyl ester) for the C-terminus of the amino acid radicals. As used herein, any abbreviations used for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their

common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, *Biochem. 11*: 942-944 (1972)).

As used herein, treatment means any manner in which one or more of the symptoms of a disease or disorder are ameliorated or otherwise

- 5 beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein, such as use for treating hyperproliferating tissue or neovascularization mediated diseases or disorders, or diseases or disorders in which hyperproliferating tissue or neovascularization is implicated.

- As used herein, amelioration of the symptoms of a particular disorder by
10 administration of a particular compound or pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

- As used herein, a prodrug is a compound that, upon *in vivo* administration, is metabolized by one or more steps or processes or otherwise
15 converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve
20 the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound (see, e.g., Nogrady (1985) *Medicinal Chemistry A Biochemical Approach*, Oxford University Press, New
25 York, pages 388-392).

- It is to be understood that the compounds provided herein may contain chiral centers. Such chiral centers may be of either the (R) or (S) configuration, or may be a mixture thereof. Thus, the compounds provided herein may be enantiomerically pure, or be stereoisomeric or diastereomeric
30 mixtures. It is to be understood that the chiral centers of the compounds provided herein may undergo epimerization *in vivo*. As such, one of skill in the art will recognize that administration of a compound in its (R) form is

equivalent, for compounds that undergo epimerization *in vivo*, to administration of the compound in its (S) form.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard
5 methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis, high performance liquid chromatography (HPLC) and mass spectrometry (MS), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities,
10 of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

15 As used herein, "antibodies and antibody fragments" refers generally to immunoglobulins or fragments thereof that specifically bind to antigens to form immune complexes. The antibody may be whole immunoglobulin of any class, e.g., IgG, IgM, IgA, IgD, IgE, chimeric or hybrid antibodies with dual or multiple antigen or epitope specificities. It can be a polyclonal antibody, preferably an
20 affinity-purified antibody from a human or an appropriate animal, e.g., a primate, goat, rabbit, mouse or the like. Monoclonal antibodies are also suitable for use in the present invention, and are preferred because of their high specificities. They are readily prepared by what are now considered conventional procedures of immunization of mammals with immunogenic
25 antigen preparation, fusion of immune lymph or spleen cells with an immortal myeloma cell line, and isolation of specific hybridoma clones. More unconventional methods of preparing monoclonal antibodies are not excluded, such as interspecies fusions and genetic engineering manipulations of hypervariable regions, since it is primarily the antigen specificity of the
30 antibodies that affects their utility. Newer techniques for production of monoclonals can also be used, e.g., human monoclonals, interspecies

monoclonals, chimeric (e.g., human/mouse) monoclonals, genetically engineered antibodies and the like.

As used herein, an "infecting agent" denotes invading microbes or parasites. As used herein, "microbe" denotes virus, bacteria, rickettsia,

5 mycoplasma, protozoa, fungi and like microorganisms, and "parasite" denotes infectious, generally microscopic or very small multicellular invertebrates, or ova or juvenile forms thereof, which are susceptible to antibody-induced clearance or lytic or phagocytic destruction, e.g., malarial parasites, spirochetes and the like.

10 As used herein, "tumor" denotes a neoplasm, and includes both benign and malignant tumors. This term particularly includes malignant tumors which can be either solid (such as a breast, liver, or prostate carcinoma) or non-solid (such as a leukemia). Tumors can also be further divided into subtypes, such as adenocarcinomas (e.g. of the breast, prostate or lung).

15 As used herein, "a target" denotes the object that is intended to be detected, diagnosed, impaired or destroyed by the methods provided herein, and includes target cells, target tissues, and target compositions. "Target tissues" and "target cells" as used herein are those tissues that are intended to be impaired or destroyed by this treatment method. Photosensitizing
20 compounds bind to these target tissues or target cells; then when sufficient radiation is applied, these tissues or cells are impaired or destroyed. Target cells are cells in target tissue, and the target tissue includes, but is not limited to, vascular endothelial tissue, abnormal vascular walls of tumors, solid tumors such as (but not limited to) tumors of the head and neck, tumors of the eye, tumors of
25 the gastrointestinal tract, tumors of the liver, tumors of the breast, tumors of the prostate, tumors of the lung, nonsolid tumors and malignant cells of the hematopoietic and lymphoid tissue, neovascular tissue, other lesions in the vascular system, bone marrow, and tissue or cells related to autoimmune disease. Also included among target cells are cells undergoing substantially
30 more rapid division as compared to non-target cells.

"Non-target tissues" as used herein are all the tissues of the subject which are not intended to be impaired or destroyed by the treatment method.

These non-target tissues include but are not limited to healthy blood cells, and other normal tissue, not otherwise identified to be targeted.

"Target compositions" as used herein are those compositions that are intended to be impaired or destroyed by this treatment method, and may
5 include one or more pathogenic agents, including but not limited to bacteria, viruses, fungi, protozoa, and toxins as well as cells and tissues infected or infiltrated therewith. The term "target compositions" also includes, but is not limited to, infectious organic particles such as prions, toxins, peptides, polymers, and other compounds that may be selectively and specifically identified as an
10 organic target that is intended to be impaired or destroyed by this treatment method.

"Hyperproliferative tissue" as used herein means tissue that grows out of control and includes neoplastic tissue, tumors and unbridled vessel growth such as blood vessel growth found in age-related macular degeneration and
15 often occurring after glaucoma surgeries.

"Hyperproliferative disorders" as used herein denotes those conditions disorders sharing as an underlying pathology excessive cell proliferation caused by unregulated or abnormal cell growth, and include uncontrolled angiogenesis. Examples of such hyperproliferative disorders includes, but are
20 not limited to, cancers or carcinomas, acute and membrano-proliferative glomerulonephritis, myelomas, psoriasis, atherosclerosis, psoriatic arthritis, rheumatoid arthritis, diabetic retinopathies, macular degeneration, corneal neovascularization, choroidal hemangioma, recurrence of pterygii, and scarring from excimer laser surgery and glaucoma filtering surgery.

25 A "therapeutically effective dose" as used herein is a dose sufficient to prevent advancement, or to cause regression of the disease, or which is capable of relieving symptoms caused by the disease.

A "pharmaceutical agent" or "drug" refers to a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect
30 when properly administered to a subject.

All chemical compounds include both the (+) and (-) stereoisomers, as well as either the (+) or (-) stereoisomer.

"Irradiating" and "irradiation" as used herein includes exposing a subject to all wavelengths of light. Preferably, the irradiating wavelength is selected to match the wavelength(s) which excite the photosensitive compound.

Preferably, the radiation wavelength matches the excitation wavelength of the photosensitive compound and has low absorption by the non-target tissues of the subject, including blood proteins.

Irradiation is further defined herein by its coherence (laser) or non-coherence (non-laser), as well as intensity, duration, and timing with respect to dosing using the photosensitizing compound. The intensity or fluence rate must be sufficient for the light to reach the target tissue. The duration or total fluence dose must be sufficient to photoactivate enough photosensitizing compound to act on the target tissue. Timing with respect to dosing with the photosensitizing compound is important, because 1) the administered photosensitizing compound requires some time to home in on target tissue and 2) the blood level of many photosensitizing compounds decreases with time. The radiation energy is provided by an energy source, such as a laser or cold cathode light source, that is external to the subject, or that is implanted in the subject, or that is introduced into a subject, such as by a catheter, optical fiber or by ingesting the light source in capsule or pill form (*e.g.*, as disclosed in U.S. Patent 6,273,904 (2001)).

While one preferred embodiment of the present invention is drawn to the use of light energy for administering PDT to destroy tumors, other forms of energy are within the scope of this invention, as will be understood by those of ordinary skill in the art. Such forms of energy include, but are not limited to: thermal, sonic, ultrasonic, chemical, light, microwave, ionizing (such as x-ray and gamma ray), mechanical, and electrical. For example, sonodynamically induced or activated agents include, but are not limited to: gallium-porphyrin complex (see Yumita *et al.*, *Cancer Letters* 112: 79-86 (1997)), other porphyrin complexes, such as protoporphyrin and hematoporphyrin (see Umemura *et al.*, *Ultrasonics Sonochemistry* 3: S187-S191 (1996)); other cancer drugs, such as daunorubicin and adriamycin, used in the presence of ultrasound therapy (see Yumita *et al.*, *Japan J. Hyperthermic Oncology* 3(2):175-182 (1987)).

- As used herein, destroy means to kill the desired target tissue or target composition, including infecting agents. "Impair" means to change the target tissue or target composition in such a way as to interfere with its function or reduce its growth. For example, in North *et al.*, it is observed that after
- 5 virus-infected T cells treated with benzoporphyrin derivatives were exposed to light, holes developed in the T cell membrane and increased in size until the membrane completely decomposed (*Blood Cells* 18:129-40 (1992)). The target tissue or target composition is understood to be impaired or destroyed even if the target tissue or target composition is ultimately disposed of by macrophages.
- 10 The present invention provides a method for providing a medical therapy to an animal, and the term "animal" includes, but is not limited to, humans and other mammals. The term "mammals" or "mammalian subject" includes farm animals, such as cows, hogs and sheep, as well as pet or sport animals such as horses, dogs, and cats.
- 15 The term "coupling agent" as used herein, refers to a reagent capable of coupling a photosensitizer to a targeting agent
- As used herein, "targeting agent" refers to a compound that homes in on or preferentially associates or binds to a particular tissue, receptor, infecting agent or other area of the body of the subject to be treated, such as a target
- 20 tissue or target composition. Examples of a targeting agent include but are not limited to a ligand, one member of a ligand-receptor binding pair, and liposomal suspensions, including tissue-targeted liposomes.
- As used herein, "specific binding pair" and "ligand-receptor binding pair" refers to two different molecules, where one of the molecules has an
- 25 area on the surface or in a cavity which specifically attracts or binds to a particular spatial or polar organization of the other molecule, causing both molecules to have an affinity for each other. The members of the specific binding pair are referred to as ligand and receptor (anti-ligand). The terms ligand and receptor are intended to encompass the entire ligand or receptor or
- 30 portions thereof sufficient for binding to occur between the ligand and the receptor. Examples of ligand-receptor binding pairs include, but are not limited to, hormones and hormone receptors, for example epidermal growth factor

and epidermal growth factor receptor, tumor necrosis factor- α and tumor necrosis factor-receptor, and interferon and interferon receptor; avidin and biotin or antibiotin; antibody and antigen pairs; enzymes and substrates, drug and drug receptor; cell-surface antigen and lectin; two complementary nucleic acid strands; nucleic acid strands and complementary oligonucleotides; interleukin and interleukin receptor; and stimulating factors and their receptors, such as granulocyte-macrophage colony stimulating factor (GM-CSF) and GM-CSF receptor and macrophage colony stimulating factor (M-CSF) and M-CSF receptor.

As used herein, the term "bind" or "binding" is used to refer to the binding between a targeting agent and its target, such as a ligand and its receptor, with a K_d in the range of 10^{-2} to 10^{-15} mole/l, generally, 10^{-6} to 10^{-15} , 10^{-7} to 10^{-15} and typically 10^{-8} to 10^{-15} (and/or a K_a (binding affinity) of 10^5 - 10^{12} , 10^7 - 10^{12} , 10^8 - 10^{12} l/mole).

As used herein, specific binding or selective binding means that the binding of a targeting agent with its target, such as a particular ligand and its receptor is at least 2-fold, generally, 5, 10, 50, 100 or more-fold, greater than for non-target, such as another receptor. A statement that a particular compound is targeted to a target cell or target tissue means that its affinity for such cell or tissue in a host or *in vitro* or *in vivo* is at least about 2-fold, generally, 5, 10, 50, 100 or more-fold, greater than for other cells and tissues in the host or under the *in vitro* conditions.

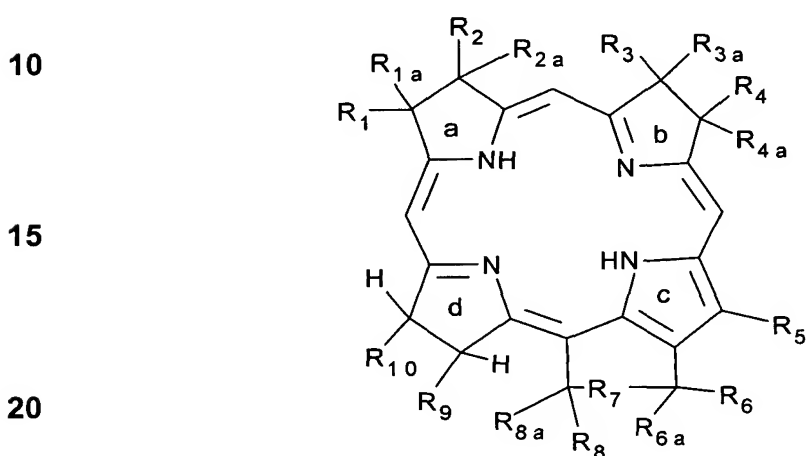
As used herein, sample refers to anything that contains an target for which a target assay is desired. The sample can be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, sperm, amniotic fluid or the like. Biological tissues are aggregates of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

As used herein, combination refers to any association between 2 or more items.

B. COMPOUNDS

5 Provided herein are tetrapyrrole compounds containing a fluorinated substituent where the compound is a chlorin or bacteriochlorin.

Also provided are compounds of the formula:



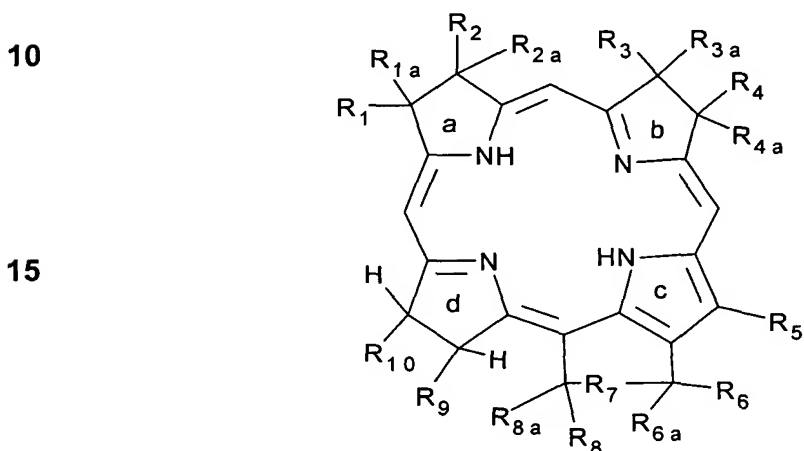
or a pharmaceutically acceptable derivative thereof, where R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_5 , R_6 , R_{6a} , R_8 , R_{8a} , R_9 , and R_{10} are independently hydrogen, lower alkyl of about 1 through 8 carbon atoms, lower alkenyl of about 1 through 8 carbon atoms, or lower alkyl of about 1 through 8 carbon atoms substituted with at least one halogen, hydroxy, carboxy, ester, aromatic, heterocyclic, ether, amide, or amine group; where two R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{4a} , R_6 , R_{6a} , R_8 , R_{8a} , R_9 and R_{10} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_6 , R_{6a} , R_8 , and R_{8a} groups on the same carbon atom may form a double bond to a divalent pendant group; R_1 or R_2 may additionally be $-\text{CH}=\text{CH}_2$, $-\text{CHO}$, $-\text{COOH}$, $-\text{COOR}_a$, or $\text{H}_3\text{C}-\text{OR}_{11}$; R_7 is $-\text{CH}_2-$, or $=\text{NR}_{12}$, or a covalent bond, where R_{11} , and R_{12} are independently hydrogen, lower alkyl of about 1 through 8 carbon atoms, lower alkenyl of about 1 through 8 carbon atoms, or lower alkyl of about 1 through 8 carbon atoms substituted with at least one

halogen, hydroxy, carboxy, ester, aromatic, heterocyclic, ether, amide, or amine group; provided that at least one of R_1 , R_{1a} , R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_{4a} , R_5 , R_6 , R_{6a} , R_7 , R_8 , R_{8a} , R_9 and R_{10} contains at least one fluorinated pendant group selected from the group consisting of fluorinated alkyl groups,

5 fluorinated phenyl groups and fluorinated heterocyclic moieties.

Also provided are compounds where R_9 is $-\text{CH}_2\text{CH}_2\text{CO}_2\text{R}_a$, where R_a is hydrogen or lower alkyl of 1-8 carbons.

Also provided are compound of the formula:



20 or a pharmaceutically acceptable derivative thereof, where

R_1 and R_2 are each independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, $-\text{C}(\text{O})\text{R}_a$ or $-\text{COOR}_a$, where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted

25 cycloalkyl;

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl;

30 R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_5 is hydrogen or substituted or unsubstituted alkyl;

R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form $=\text{O}$;

R_7 is a covalent bond, alkylene, azaalkyl, or azaaralkyl;

R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form =O;

R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl;

each of R_1 - R_{10} , when substituted, is substituted with one or more substituents, in one embodiment one to five substituents, in another embodiment one, two or three substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue;

each Q is independently unsubstituted or is substituted with one or more substituents, in one embodiment one to five substituents, in another embodiment one, two or three substituents, each independently selected from Q_1 , where Q_1 is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue;

with the proviso that the compound contains at least one fluorine atom.

Also provided are compounds where

R_1 is substituted or unsubstituted alkyl;

R_2 is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or $-C(O)R_a$, where R_a is substituted or unsubstituted alkyl;

R_{1a} and R_{2a} together form a covalent bond;

- R_3 and R_4 are each independently substituted or unsubstituted alkyl;
 R_{3a} and R_{4a} are each independently hydrogen, or together form a covalent bond;
- R_5 is substituted or unsubstituted alkyl;
- 5 R_6 and R_{6a} together form $=O$;
- R_7 is azaalkyl, or azaaralkyl;
- R_8 and R_{8a} together form $=O$;
- R_9 and R_{10} are each independently substituted or unsubstituted alkyl;
- each of R_1 - R_{10} , when substituted, is substituted with one or more
- 10 substituents each independently selected from Q, where Q is halo, pseudohalo, haloalkyl, $COOR_b$ where R_b is hydrogen or alkyl, OR_c where R_c is alkyl or aralkyl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl or aralkyl, or $=NR_h$ where R_h is aralkyl;
- each Q is independently unsubstituted or is substituted with one or
- 15 more substituents each independently selected from Q_1 , where Q_1 is halo, pseudohalo, or haloalkyl.
- Also provided are compounds where R_1 is unsubstituted alkyl;
- R_2 is substituted or unsubstituted alkyl, unsubstituted alkenyl, or -
 $C(O)R_a$, where R_a is unsubstituted alkyl;
- 20 R_{1a} and R_{2a} together form a covalent bond;
- R_3 and R_4 are each independently unsubstituted alkyl;
- R_{3a} and R_{4a} are each independently hydrogen, or together form a covalent bond;
- R_5 is unsubstituted alkyl;
- 25 R_6 and R_{6a} together form $=O$;
- R_7 is azaalkyl, or azaaralkyl;
- R_8 and R_{8a} together form $=O$;
- R_9 is substituted alkyl;
- R_{10} is unsubstituted alkyl;
- 30 each of R_1 - R_{10} , when substituted, is substituted with one or more substituents each independently selected from Q, where Q is halo, pseudohalo, haloalkyl, $COOR_b$ where R_b is hydrogen or alkyl, OR_c where R_c is

alkyl or aralkyl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl or aralkyl, or $=\text{NR}_h$ where R_h is aralkyl;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1 , where Q_1 is halo, 5 pseudohalo, or haloalkyl.

Further provided are compounds where R_1 is methyl;

R_{1a} and R_{2a} together form a covalent bond;

R_3 is methyl; R_4 is ethyl;

R_{3a} and R_{4a} are each independently hydrogen, or together form a 10 covalent bond;

R_5 is methyl;

R_9 is $\text{CH}_2\text{CH}_2\text{COOH}$ or $\text{CH}_2\text{CH}_2\text{COOMe}$;

R_{10} is methyl.

Also provided are compounds where R_2 is $\text{CH}=\text{CH}_2$, $\text{CH}(\text{OR}_{20})\text{CH}_3$, 15 $\text{C}(\text{O})\text{Me}$, $\text{C}(=\text{NR}_{21})\text{CH}_3$ or $\text{CH}(\text{NHR}_{21})\text{CH}_3$;

where R_{20} is methyl, butyl, heptyl, dodecyl or 3,5-bis(trifluoromethyl)-benzyl; and

R_{21} is 3,5-bis(trifluoromethyl)benzyl.

Also provided are compounds where R_7 is $=\text{NR}_{20}$, where R_{20} is methyl, 20 butyl, heptyl, dodecyl or 3,5-bis(tri-fluoromethyl)benzyl.

In another embodiment, R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; $-\text{C}(\text{O})\text{R}_a$ or $-\text{COOR}_a$, where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted 25 alkenyl.

In another embodiment, R_2 is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; $-\text{C}(\text{O})\text{R}_a$ or $-\text{COOR}_a$, where R_a is hydrogen, substituted or unsubstituted alkyl, or substituted or unsubstituted 30 alkenyl.

In a further embodiment, R_{1a} is hydrogen or substituted or unsubstituted alkyl.

In another embodiment, R_{2a} is hydrogen or substituted or unsubstituted alkyl.

In a further embodiment, R_{1a} and R_{2a} together form a covalent bond.

In another embodiment, R_3 is hydrogen or substituted or unsubstituted
5 alkyl;

In a further embodiment, R_4 is hydrogen or substituted or unsubstituted alkyl;

In another embodiment, R_{3a} is hydrogen.

In another embodiment, R_{4a} is hydrogen.

10 In a further embodiment, R_{3a} and R_{4a} together form a covalent bond.

In another embodiment, R_5 is substituted or unsubstituted alkyl.

In another embodiment, R_6 and R_{6a} together form $=O$.

In a further embodiment, R_7 is azaalkyl or azaaralkyl.

In another embodiment, R_8 and R_{8a} together form $=O$.

15 In another embodiment, R_9 is substituted alkyl.

In a further embodiment, R_{10} is unsubstituted alkyl.

In another embodiment, each of R_1 - R_{10} is substituted with one to five substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl,

20 cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl,
25 alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is unsubstituted.

In a further embodiment each of R_1 - R_{10} is substituted with one substituent selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl,

30 heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where

R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is unsubstituted.

- In another embodiment, each of R_1 - R_{10} is substituted with two
- 5 substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl
- 10 or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is unsubstituted.

- In a further embodiment each of R_1 - R_{10} is substituted with three
- 15 substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl
- 20 or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is unsubstituted.

- In another embodiment, each of R_1 - R_{10} is substituted with one to five
- 25 substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, $-COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl
- 30 or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=NR_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is

independently substituted with one to five substituents selected from Q_1 , where Q_1 is alkyl, haloalkyl, halo, pseudohalo, $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue.

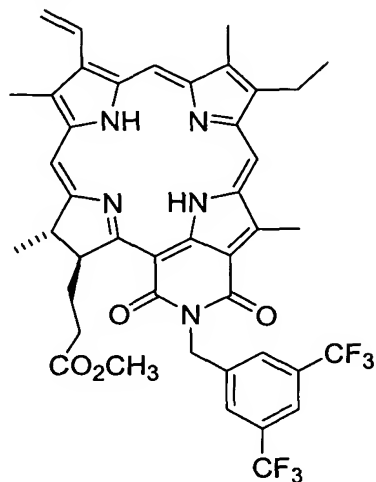
In a further embodiment each of R_1 - R_{10} is substituted with one substituent selected from Q , where Q is alkyl, haloalkyl, halo, pseudohalo, $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=\text{NR}_h$ where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is independently substituted with one substituent selected from Q_1 , where Q_1 is alkyl, haloalkyl, halo, pseudohalo, $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue.

In another embodiment, each of R_1 - R_{10} is substituted with two substituents, each independently selected from Q , where Q is alkyl, haloalkyl, halo, pseudohalo, $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, $=\text{NR}_h$ where R_h is alkyl, alkenyl,

alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is substituted with two substituents, each independently selected from Q₁, where Q₁ is alkyl, haloalkyl, halo, pseudohalo, -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue.

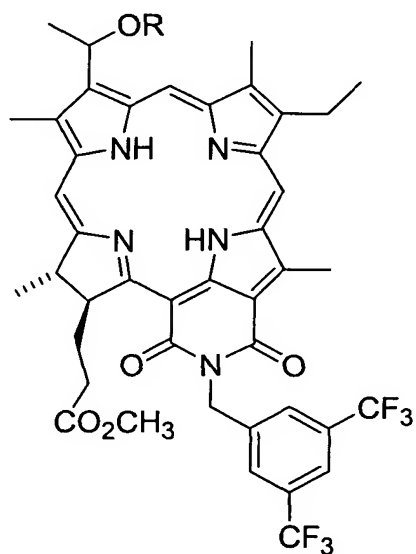
In a further embodiment each of R₁-R₁₀ is substituted with three substituents, each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl, or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, =NR_h where R_h is alkyl, alkenyl, alkynyl, cycloalkyl, aralkyl or aryl, or is an amino acid residue, where each Q is substituted with three substituents, each independently selected from Q₁, where Q₁ is alkyl, haloalkyl, halo, pseudohalo, -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, aryl, heteroaryl, cycloalkyl, heterocyclyl, OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or aryl, or is an amino acid residue.

Also provided is a compound of the formula:



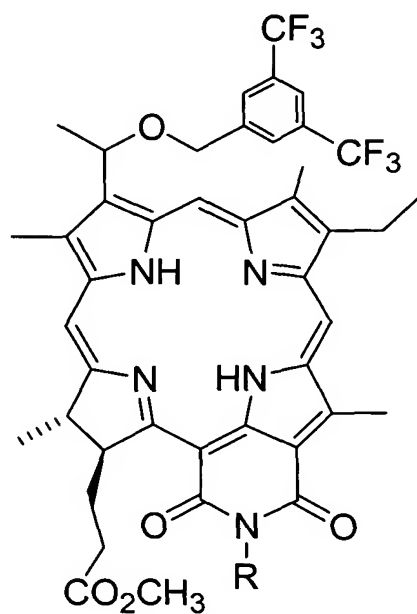
or a pharmaceutically acceptable derivative thereof.

5 Also provided is a compound of the formula:



or a pharmaceutically acceptable derivative thereof, where R is methyl, butyl, heptyl or dodecyl.

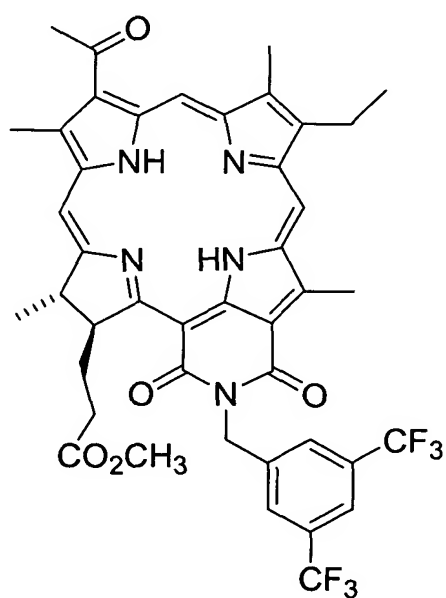
Also provided is a compound of the formula:



or a pharmaceutically acceptable derivative thereof, where R is methyl, butyl, heptyl or dodecyl.

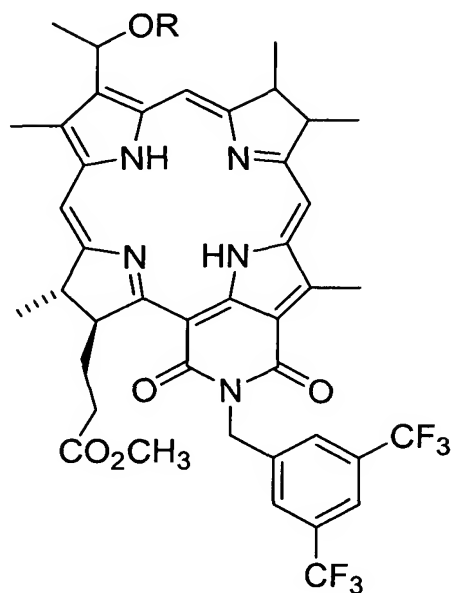
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Also provided is a compound of the formula:



or a pharmaceutically acceptable derivative thereof.

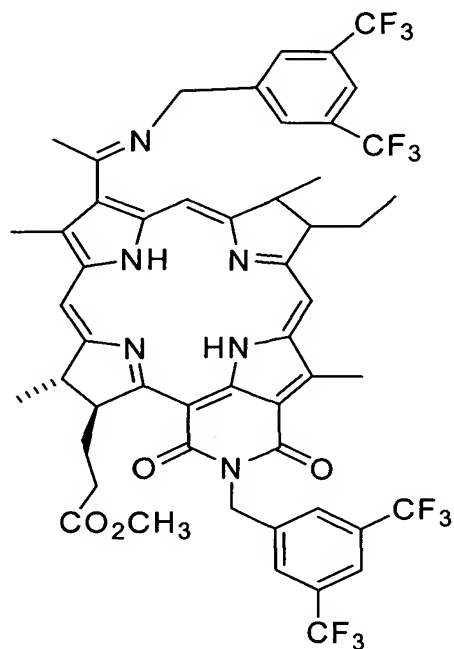
Also provided is a compound of the formula:



or a pharmaceutically acceptable derivative thereof, where R is methyl, butyl, heptyl or dodecyl.

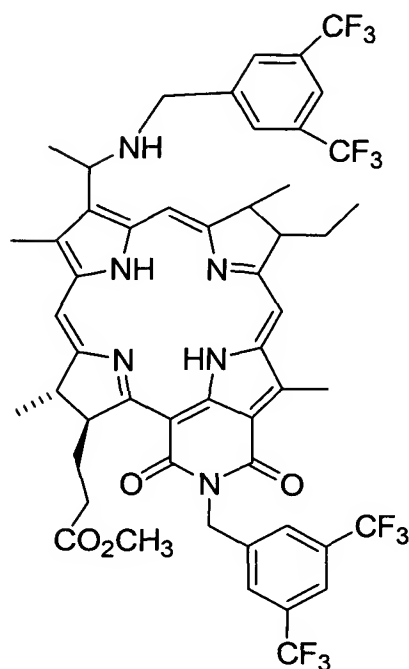
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Also provided is a compound of the formula:



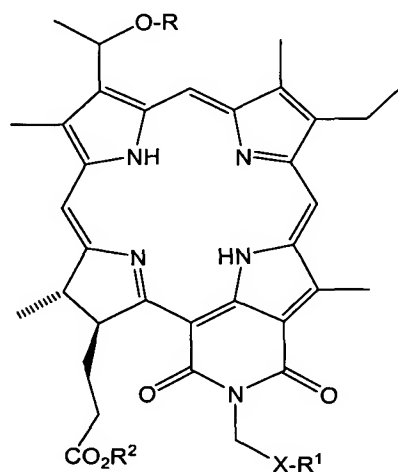
or a pharmaceutically acceptable derivative thereof.

Also provided is a compound of the formula:



or a pharmaceutically acceptable derivative thereof.

Also provided is a compound of the formula

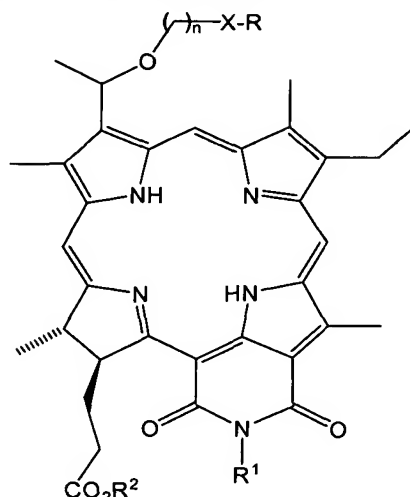


5

or a pharmaceutically acceptable derivative thereof, where X is an aryl or heteroaryl group; R and R¹ are each independently alkyl, aryl, or heteroaryl groups having 1 – 20 carbon atoms, wherein at least one of R and R¹ is substituted with at least one fluorine atom; and R² is an alkyl group, optionally substituted with one or more fluorine atoms.

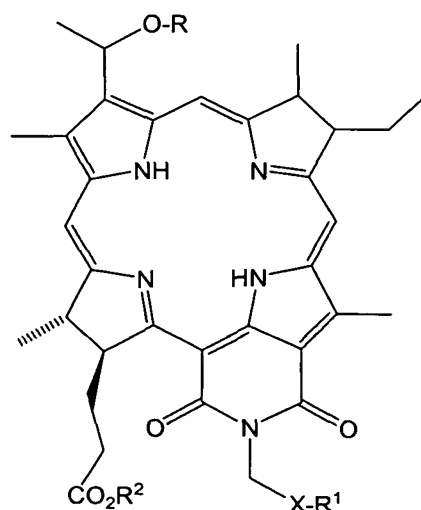
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Also provided is a compound of the formula



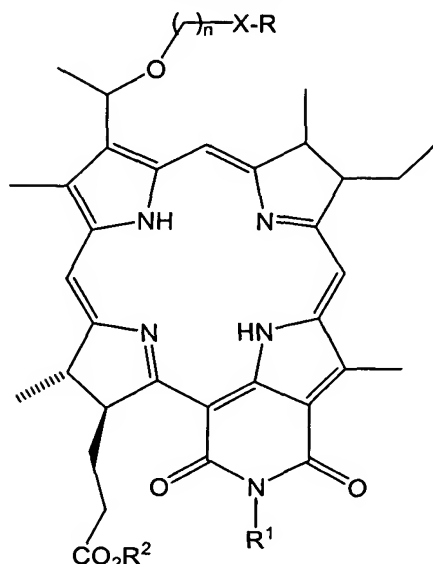
or a pharmaceutically acceptable derivative thereof, where X is an aryl or heteroaryl group; n is an integer from 0 to 6; R and R¹ are each independently alkyl, aryl, or heteroaryl groups having 1 – 20 carbon atoms, where at least one of R and R¹ is substituted with at least one fluorine atom; and R² is an alkyl group, optionally substituted with one or more fluorine atoms.

Also provided is a compound of the formula



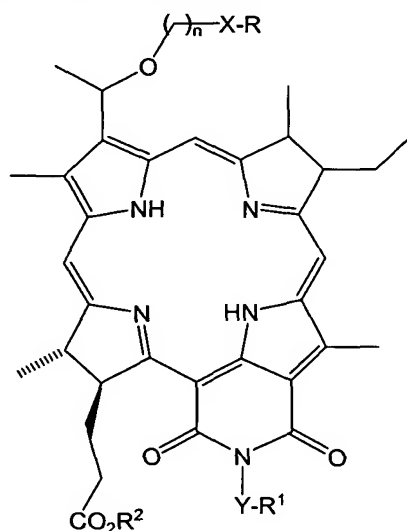
10 or a pharmaceutically acceptable derivative thereof, where X is an aryl or heteroaryl group; R and R¹ are each independently alkyl, aryl, or heteroaryl groups having 1 – 20 carbon atoms, where at least one of R and R¹ is substituted with at least one fluorine atom; and R² is an alkyl group, optionally substituted with one or more fluorine atoms.

Also provided is a compound of the formula



- or a pharmaceutically acceptable derivative thereof, where X is an aryl or heteroaryl group; n is an integer from 0 to 6; R and R¹ are each independently alkyl, aryl, or heteroaryl groups having 1 – 20 carbon atoms, wherein at least one of R and R¹ is substituted with at least one fluorine atom; and R² is an alkyl group, optionally substituted with one or more fluorine atoms.

Also provided is a compound of the formula



- 10 or a pharmaceutically acceptable derivative thereof, where X and Y are each independently an aryl or heteroaryl group; n is an integer from 0 to 6; R and R¹ are each independently alkyl, aryl, or heteroaryl groups having 1 – 20 carbon

atoms, wherein at least one of R and R¹ is substituted with at least one fluorine atom; and R² is an alkyl group, optionally substituted with one or more fluorine atoms.

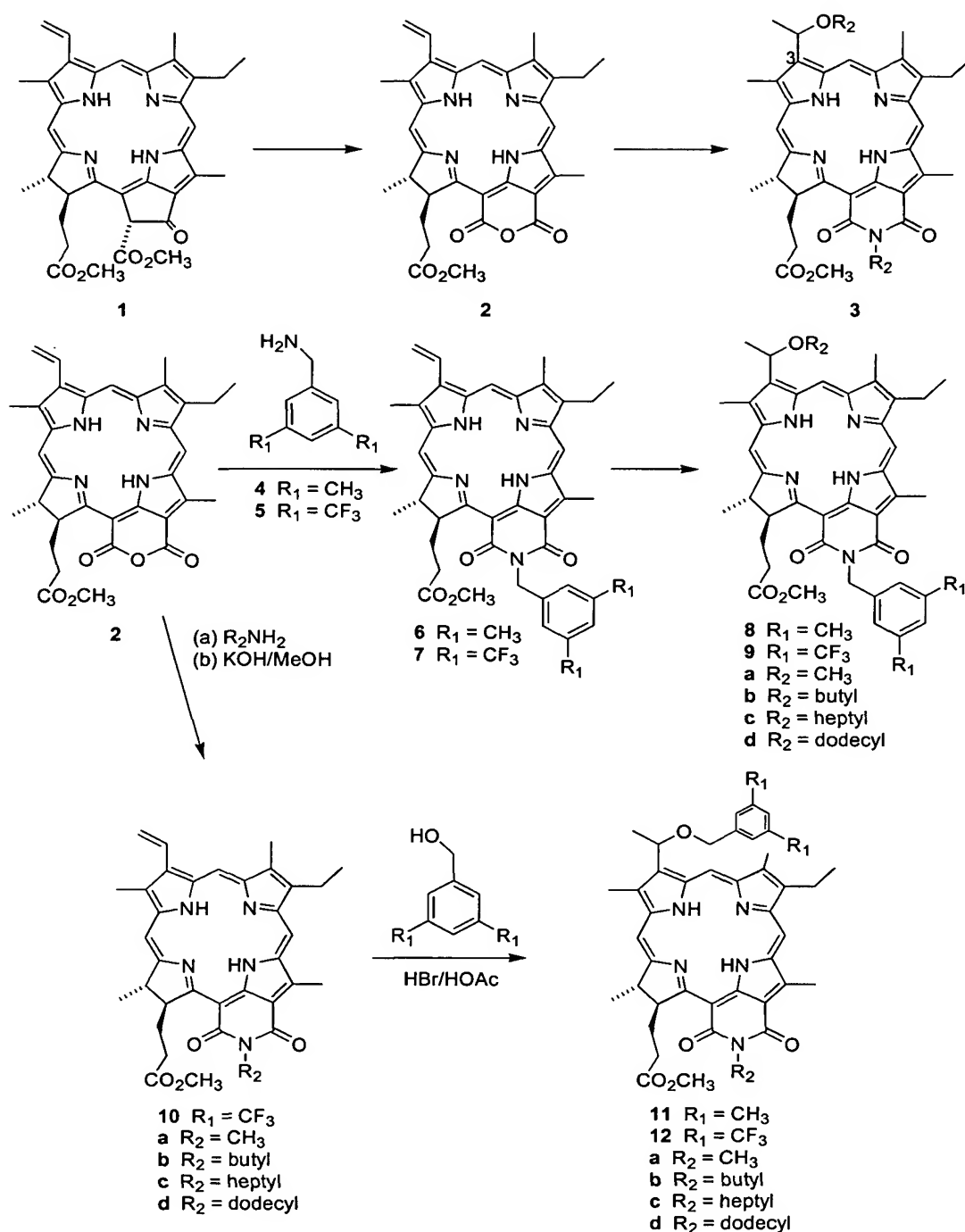
C. PREPARATION OF THE COMPOUNDS

5

The compounds provided herein may be prepared according to the methods provided below and exemplified herein (see, *e.g.*, EXAMPLE 3). The starting materials for synthesis of the compounds are readily available from commercial sources (*e.g.*, Aldrich Chemical Company, Milwaukee, WI, USA).

- 10 Other compounds within the scope of the instant disclosure may be prepared by routine modification of the procedures provided herein using appropriate starting materials, the selection of which will be evident to those of skill in the art.

- 15 In order to determine the effect of the presence and position of aromatic substituents (instead of N-alkyl and or O-alkyl) on biological activity, purpurinimides **8** and **11** containing N-3,5-dimethylbenzyl- or 3-[1'-(3,5-dimethyl benzyloxy) ethyl] substituents with similar log P value (11.83) were synthesized, as shown in Scheme 1. This approach was then extended for the preparation of the related N- and O-trifluoromethyl substituted analogs **9** and
- 20 **12**, respectively. In brief, methylpheophorbide-*a* **1** was extracted from *Spirulina pacifica* and converted into purpurin-18 methyl ester **2** by following well established methodology (Smith, K. M.; Goff, D. A. and Simpson, D., *J. Am. Chem. Soc.* **107**: 4941 (1985); Smith, K. M., *Porphyrins and Metalloporphyrins* (Smith, K. M., Ed.), Elsevier Sci. Pub, Amsterdam, 1975). Reaction
- 25 of **2** with 3,5-dimethyl-benzyl amine at room temperature produced the intermediate amide as a mixture of two isomers, which under base-catalyzed intramolecular cyclization produced purpurinimide **6** in 70% overall yield. Further reaction of **6** with HBr/HOAc and then with 1-butanol gave the corresponding n-butyl ether derivative **8** in 70% yield. By following similar
- 30 reaction conditions, other related non-fluorinated and fluorinated photosensitizers **9**, **11** and **12** were also prepared, as shown in Scheme 1.



Scheme 1

The structures of these compounds were confirmed by NMR and mass spectrometry analyses (Zheng, G.; Potter, W. R.; Camacho, S. H., Missert, J. R., Wang, G., Bellnier, D. A., Henderson, B. W., Rodgers, M. A. J., Dougherty, T. J., and Pandey, R. K., *J. Med. Chem.* 44: 1540-1559 (2001). The presence of trifluoromethyl substituents in purpurinimides 9 and 12 was confirmed by ^{19}F

NMR spectroscopy (the resonances for the symmetrical trifluoromethyl groups in **9** and **12** were observed at δ 13.145 and 12.598 ppm, respectively).

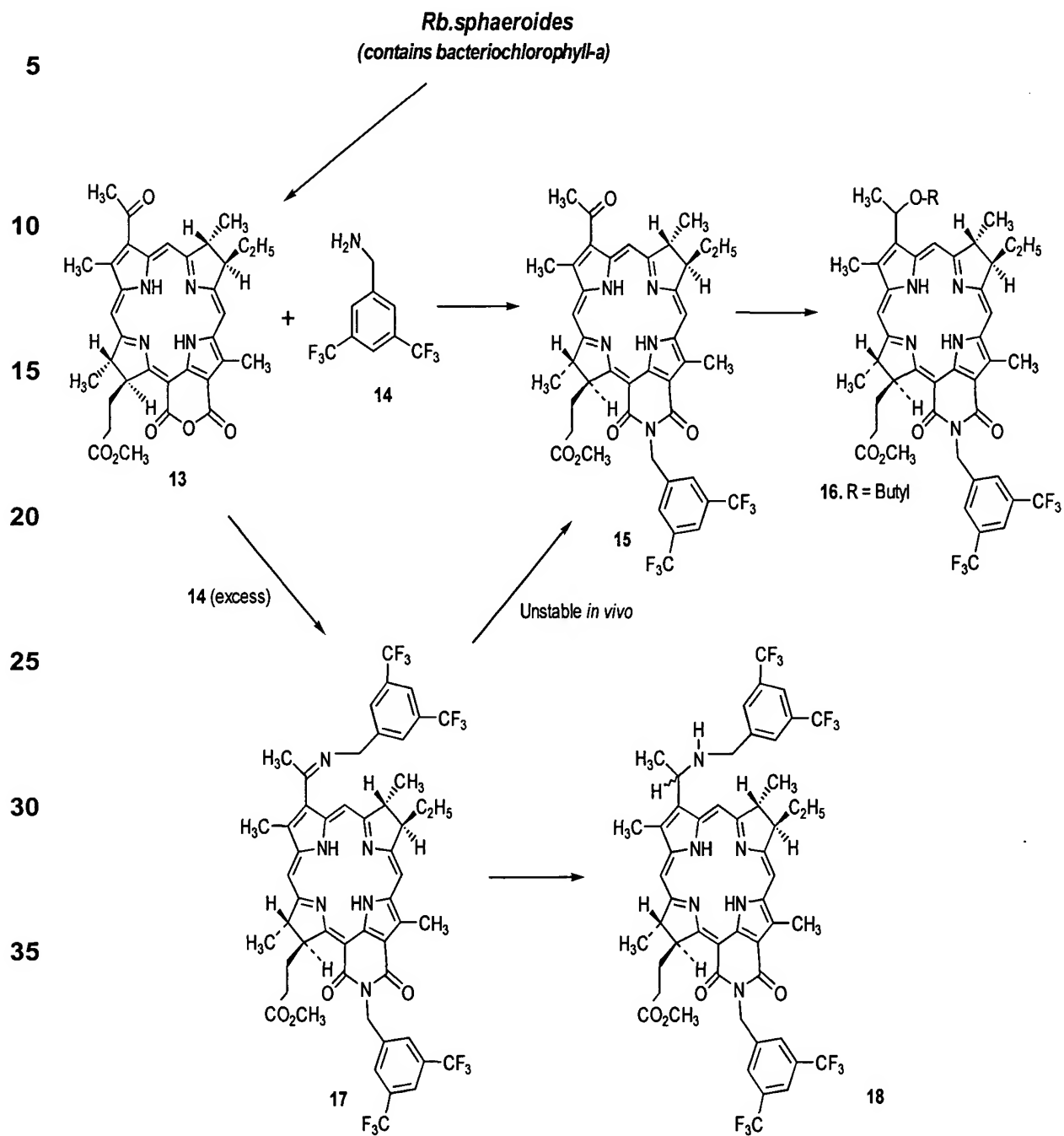
The electronic absorption spectra of PHOTOFRIN[®] (a porphyrin analog), HPPH (a chlorin bearing a five-membered isocyclic ring) and the purpurinimide **9** (a chlorin with an expanded, six-membered fused imide ring) were measured in dichloromethane at the same molar concentrations. As can be seen from FIGURE 1, both HPPH (λ_{\max} = 660 nm) and purpurinimide (λ_{\max} = 700 nm) produce stronger long wavelength absorption (ϵ = 45,000) than the porphyrin PHOTOFRIN[®] with considerable red-shifts of 30 nm and 70 nm, respectively.

Compounds Related to Bacteriochlorins

As discussed above, the studies with a series of purpurinimides indicate that among all the photosensitizers evaluated so far, the purpurinimide **9** containing N-[(3,5-bis(trifluoromethyl) benzyl] group produced the best *in vivo* efficacy. Therefore, to investigate the effect of such substituents in compounds with longer wavelength absorption near 800 nm, bacteriopurpurin-18 methyl ester **13** (a bacteriochlorin) obtained by following the methodology developed in our laboratory (see Scheme 2 below and U.S. Patent No. 5,591,847) was treated with 3,5-bis(trifluoromethyl) benzyl amine.

The formation of the reaction products was found to be temperature dependent. For example, when the reaction was performed at elevated temperature, the N-fluorinated derivative **15** and the corresponding Schiff base **17** (obtained from the reaction of the 3-acetyl group with an amine) was isolated in almost equal ratio. However, at low temperature, 3-acetyl-N-substituted-bacteriopurpurinimide **15** was isolated as a major product. Bacteriochlorin **15** on reaction with sodium borohydride and then with HBr gas followed by a reaction with n-butyl alcohol produced the corresponding butyl ether derivative **16**. The Schiff base **17** was found to be unstable *in vivo* and rapidly converted into the related acetyl analog **15** in quantitative yield (determined by *in vivo* reflectance spectroscopy). However, reduction of **17**

with sodium borohydride at room temperature produced stable amine **18**. See Scheme 2.



Scheme-2

D. PHARMACEUTICAL COMPOSITIONS

1. Formulation of pharmaceutical compositions

The pharmaceutical compositions provided herein contain

- 5 therapeutically effective amounts of one or more of the compounds provided herein that are useful in the prevention, treatment, or amelioration of one or more of the symptoms of diseases or disorders associated with hyperproliferating tissue or neovascularization, or in which hyperproliferating tissue or neovascularization is implicated, in a pharmaceutically acceptable
- 10 carrier. Diseases or disorders associated with hyperproliferating tissue or neovascularization include, but are not limited to, cancer, psoriasis, atherosclerosis, heart disease, and age-related macular degeneration. Pharmaceutical carriers suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable
- 15 for the particular mode of administration.

In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

The compositions contain one or more compounds provided herein.

- 20 The compounds are, in one embodiment, formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration or in sterile solutions or suspensions for parenteral administration, as well as transdermal patch preparation and dry
- 25 powder inhalers. In one embodiment, the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see, e.g., Ansel, *Introduction to Pharmaceutical Dosage Forms, Fourth Edition* 1985, 126).

- In the compositions, effective concentrations of one or more
- 30 compounds or pharmaceutically acceptable derivatives thereof is (are) mixed with a suitable pharmaceutical carrier. The compounds may be derivatized as the corresponding salts, esters, enol ethers or esters, acetals, ketals,

orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs prior to formulation, as described above. The concentrations of the compounds in the compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the

5 symptoms of diseases or disorders associated with hyperproliferating tissue or neovascularization or in which hyperproliferating tissue or neovascularization is implicated.

In one embodiment, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of compound is

10 dissolved, suspended, dispersed or otherwise mixed in a selected carrier at an effective concentration such that the treated condition is relieved, prevented, or one or more symptoms are ameliorated.

The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the

15 absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in *in vitro* and *in vivo* systems described herein and in U.S Patent 5,952,366 to Pandey *et al.* (1999) (see, e.g., EXAMPLE 7) and then extrapolated therefrom for dosages for humans.

20 The concentration of active compound in the pharmaceutical composition will depend on absorption, inactivation and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, the amount that is delivered is sufficient

25 to ameliorate one or more of the symptoms of diseases or disorders associated with hyperproliferating tissue or neovascularization or in which hyperproliferating tissue or neovascularization is implicated, as described herein.

In one embodiment, a therapeutically effective dosage should produce

30 a serum concentration of active ingredient of from about 0.1 ng/ml to about 50-100 µg/ml. The pharmaceutical compositions, in another embodiment, should provide a dosage of from about 0.001 mg to about 2000 mg of compound per

kilogram of body weight per day. Pharmaceutical dosage unit forms are prepared to provide from about 0.01 mg, 0.1 mg or 1 mg to about 500mg, 1000 mg or 2000 mg, and in one embodiment from about 10 mg to about 500 mg of the active ingredient or a combination of essential ingredients per

5 dosage unit form.

The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known
10 testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person
15 administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known
20 to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as TWEEN[®], or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions.

25 Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms
30 of the disease, disorder or condition treated and may be empirically determined.

The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are, in one embodiment, formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975.

- 5 Dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001%-100% active ingredient, in one embodiment 0.1-95%, in another embodiment 75-
10 85%.

2. Compositions for oral administration

- Oral pharmaceutical dosage forms are either solid, gel or liquid. The solid dosage forms are tablets, capsules, granules, and bulk powders. Types of oral tablets include compressed, chewable lozenges and tablets which may
15 be enteric-coated, sugar-coated or film-coated. Capsules may be hard or soft gelatin capsules, while granules and powders may be provided in non-effervescent or effervescent form with the combination of other ingredients known to those skilled in the art.

a. Solid compositions for oral administration

- 20 In certain embodiments, the formulations are solid dosage forms, in one embodiment, capsules or tablets. The tablets, pills, capsules, troches and the like can contain one or more of the following ingredients, or compounds of a similar nature: a binder; a lubricant; a diluent; a glidant; a disintegrating agent; a coloring agent; a sweetening agent; a flavoring agent; a wetting agent; an
25 emetic coating; and a film coating. Examples of binders include microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, molasses, polyvinylpyrrolidone, povidone, crospovidones, sucrose and starch paste. Lubricants include talc, starch, magnesium or calcium stearate, lycopodium and stearic acid. Diluents include, for example,
30 lactose, sucrose, starch, kaolin, salt, mannitol and dicalcium phosphate. Glidants include, but are not limited to, colloidal silicon dioxide. Disintegrating

agents include crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose. Coloring agents include, for example, any of the approved certified water soluble FD and C dyes, mixtures thereof; and water insoluble FD and C dyes suspended on alumina hydrate. Sweetening agents include sucrose, lactose, mannitol and artificial sweetening agents such as saccharin, and any number of spray dried flavors. Flavoring agents include natural flavors extracted from plants such as fruits and synthetic blends of compounds which produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene laural ether. Emetic-coatings include fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate phthalates. Film coatings include hydroxyethylcellulose, gellan gum, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

The compound, or pharmaceutically acceptable derivative thereof, could be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H₂ blockers, and diuretics. The active

ingredient is a compound or pharmaceutically acceptable derivative thereof as described herein. Higher concentrations, up to about 98% by weight of the active ingredient may be included.

In all embodiments, tablets and capsules formulations may be coated
 5 as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient. Thus, for example, they may be coated with a conventional enterically digestible coating, such as phenylsalicylate, waxes and cellulose acetate phthalate.

b. Liquid compositions for oral administration

10 Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Aqueous solutions include, for example, elixirs and syrups. Emulsions are either oil-in-water or water-in-oil.

15 Elixirs are clear, sweetened, hydroalcoholic preparations. Pharmaceutically acceptable carriers used in elixirs include solvents. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may contain a preservative. An emulsion is a two-phase system in which one liquid is dispersed in the form of small globules throughout another liquid.
 20 Pharmaceutically acceptable carriers used in emulsions are non-aqueous liquids, emulsifying agents and preservatives. Suspensions use pharmaceutically acceptable suspending agents and preservatives. Pharmaceutically acceptable substances used in non-effervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners
 25 and wetting agents. Pharmaceutically acceptable substances used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of carbon dioxide. Coloring and flavoring agents are used in all of the above dosage forms.

Solvents include glycerin, sorbitol, ethyl alcohol and syrup. Examples
 30 of preservatives include glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol. Examples of non-aqueous liquids utilized in emulsions include mineral oil and cottonseed oil. Examples of emulsifying

agents include gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate. Suspending agents include sodium carboxymethylcellulose, pectin, tragacanth, xanthan gum, Veegum and acacia. Sweetening agents include sucrose, syrups, glycerin and artificial sweetening agents such as saccharin. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene lauryl ether. Organic acids include citric and tartaric acid. Sources of carbon dioxide include sodium bicarbonate and sodium carbonate. Coloring agents include any of the approved certified water soluble FD and C dyes, and mixtures thereof. Flavoring agents include natural flavors extracted from plants such fruits, and synthetic blends of compounds which produce a pleasant taste sensation.

For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is in one embodiment encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patent Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, *e.g.*, for example, in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, *e.g.*, water, to be easily measured for administration.

Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the active compound or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (*e.g.*, propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include those set forth in U.S. Patent Nos. RE28,819 and 4,358,603. Briefly, such formulations include, but are not limited to, those containing a compound provided herein, a dialkylated mono- or poly-alkylene glycol, including, but not limited to, 1,2-dimethoxymethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether wherein 350, 550 and 750 refer to the approximate average molecular weight of the polyethylene glycol, and one or more

antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone, hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, thiodipropionic acid and its esters, and

5 dithiocarbamates.

Other formulations include, but are not limited to, aqueous alcoholic solutions including a pharmaceutically acceptable acetal. Alcohols used in these formulations are any pharmaceutically acceptable water-miscible solvents having one or more hydroxyl groups, including, but not limited to,

10 propylene glycol and ethanol. Acetals include, but are not limited to, di(lower alkyl) acetals of lower alkyl aldehydes such as acetaldehyde diethyl acetal.

3. Injectables, solutions and emulsions

Parenteral administration, in one embodiment characterized by injection, either subcutaneously, intramuscularly or intravenously is also

15 contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In

20 addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

25 Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e.g., U.S. Patent No. 3,710,795) is also contemplated herein. Briefly, a compound provided herein is dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized

30 polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic

polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer, that is insoluble in body fluids. The compound diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

Parenteral administration of the compositions includes intravenous, subcutaneous and intramuscular administrations. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil.

- 5 Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and
- 10 dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, xanthan gum, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of
- 15 metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

- The concentration of the pharmaceutically active compound is adjusted
- 20 so that an injection provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art.

- The unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration
- 25 must be sterile, as is known and practiced in the art.

- Illustratively, intravenous or intraarterial infusion of a sterile aqueous solution containing an active compound is an effective mode of administration. Another embodiment is a sterile aqueous or oily solution or suspension containing an active material injected as necessary to produce the desired
- 30 pharmacological effect.

Injectables are designed for local and systemic administration. In one embodiment, a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, in certain embodiments more than 1% w/w of the active compound to the treated
5 tissue(s).

The compound may be suspended in micronized or other suitable form or may be derivatized to produce a more soluble active product or to produce a prodrug. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the
10 compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the condition and may be empirically determined.

4. Lyophilized powders

Of interest herein are also lyophilized powders, which can be
15 reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

The sterile, lyophilized powder is prepared by dissolving a compound provided herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. The solvent may contain an excipient which improves the
20 stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer
25 known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple
30 dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

Reconstitution of this lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected compound. Such amount can be
5 empirically determined.

5. Topical administration

Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments,
10 emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

The compounds or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see,
15 e.g., U.S. Patent Nos. 4,044,126; 4,414,209; and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier
20 such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

The compounds may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the
25 eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients can
30 also be administered. These solutions, particularly those intended for ophthalmic use, may be formulated as 0.01% - 10% isotonic solutions, pH about 5-7, with appropriate salts.

6. Compositions for other routes of administration

Other routes of administration, such as transdermal patches, including iontophoretic and electrophoretic devices, and rectal administration, are also contemplated herein.

5 Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983; 6,261,595; 6,256,533; 6,167,301; 6,024,975; 6,010,715; 5,985,317; 5,983,134; 5,948,433 and 5,860,957.

For example, pharmaceutical dosage forms for rectal administration are
10 rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles
15 and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may
20 be prepared either by the compressed method or by molding. The weight of a rectal suppository, in one embodiment, is about 2 to 3 gm.

Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

25 7. Targeted Formulations

The compounds provided herein, or pharmaceutically acceptable derivatives thereof, may also be formulated to be targeted to a particular tissue, receptor, infecting agent or other area of the body of the subject to be treated. Many such targeting methods are well known to those of skill in the
30 art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, e.g., U.S. Patent Nos. 6,316,652; 6,274,552; 6,271,359; 6,253,872; 6,139,865;

6,131,570; 6,120,751; 6,071,495; 6,060,082; 6,048,736; 6,039,975; 6,004,534; 5,985,307; 5,972,366; 5,900,252; 5,840,674; 5,759,542 and 5,709,874.

a. Liposomes

In one embodiment, liposomal suspensions, including tissue-targeted liposomes, such as tumor-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Patent No. 4,522,811. Briefly, liposomes such as multilamellar vesicles (MLV's) may be formed by drying down egg phosphatidyl choline and brain phosphatidyl serine (7:3 molar ratio) on the inside of a flask. A solution of a compound provided herein in phosphate buffered saline lacking divalent cations (PBS) is added and the flask shaken until the lipid film is dispersed. The resulting vesicles are washed to remove unencapsulated compound, pelleted by centrifugation, and then resuspended in PBS.

b. Ligands

In another embodiment, the disclosed compounds may be targeted to specific target tissues or target compositions using ligands specific for the target tissue or target composition, for example, using ligands or ligand-receptor pairs such as antibodies and antigens. Antibodies against tumor antigens and against pathogens are known. For example, antibodies and antibody fragments which specifically bind markers produced by or associated with tumors or infectious lesions, including viral, bacterial, fungal and parasitic infections, and antigens and products associated with such microorganisms have been disclosed, inter alia, in Hansen *et al.*, U.S. Pat. No. 3,927,193 and Goldenberg, U.S. Pat. Nos. 4,331,647; 4,348,376; 4,361,544; 4,468,457; 4,444,744; 4,818,709 and 4,624,846. Antibodies against an antigen, e.g., a gastrointestinal, lung, breast, prostate, ovarian, testicular, brain or lymphatic tumor, a sarcoma or a melanoma, can be used.

A wide variety of monoclonal antibodies against infectious disease agents have been developed, and are summarized in a review by Polin, in *Eur. J. Clin. Microbiol.*, 3(5): 387-398 (1984), showing ready availability. These

include monoclonal antibodies (MAbs) against pathogens and their antigens such as the following:

Anti-bacterial MAbs

- 5 Streptococcus agalactiae
- Legionella pneumophila
- Streptococcus pyogenes
- Escherichia coli
- Neisseria gonorrhoeae
- 10 Neisseria meningitidis
- Pneumococcus
- Hemophilus influenzae B
- Treponema pallidum
- Lyme disease spirochetes
- 15 Pseudomonas aeruginosa
- Mycobacterium leprae
- Brucella abortus
- Mycobacterium tuberculosis
- Tetanus toxin

20

Anti-protozoan MAbs

- Plasmodium falciparum
- Plasmodium vivax
- 25 Toxoplasma gondii
- Trypanosoma rangeli
- Trypanosoma cruzi
- Trypanosoma rhodesiense
- Trypanosoma brucei
- 30 Schistosoma mansoni
- Schistosoma japonicum
- Mesocostoides corti
- Elmeria tenella
- Onchocerca volvulus
- 35 Leishmania tropica
- Trichinella spiralis
- theileria parva
- Taenia hydatigena
- Taenia ovis
- 40 Taenia saginata

Anti-viral MAbs

- HIV-1, -2, -3
- Hepatitis A, B, C, D
- Rabies virus
- Influenza virus
- Cytomegalovirus
- Herpes simplex I and II
- Human serum parvo-like virus
- Respiratory syncytial virus
- Varicella-Zoster virus
- Hepatitis B virus
- Measles virus
- Adenovirus
- Human T-cell leukemia viruses
- Epstein-Barr virus
- Mumps virus
- Sindbis virus
- Mouse mammary tumor virus*
- Feline leukemia virus*
- Lymphocytic choriomeningitis virus
- Wart virus
- Blue tongue virus
- Sendai virus
- Reo virus
- Polio virus
- Dengue virus
- Rubella virus
- Murine leukemia virus*

* = animal virus

Antimycoplasmal MAbs

- Acholeplasma laidlawii
- Mycoplasma arthritidis
- M. hyorhinis
- M. orale
- M. arginini
- M. pneumonia

Suitable MAbs have been developed against most of the micro-organisms (bacteria, viruses, protozoa, other parasites) responsible for the majority of infections in humans, and many have been used previously for in

vitro diagnostic purposes. These antibodies, and newer MAbs that can be generated by conventional methods, are appropriate for use as target agents with the compounds provided herein.

- MAbs against malaria parasites can be directed against the sporozoite, merozoite, schizont and gametocyte stages. Monoclonal antibodies have been generated against sporozoites (circumsporozoite antigen), and have been shown to neutralize sporozoites in vitro and in rodents (N. Yoshida *et al.*, *Science* 207: 71-73 (1980)). Monoclonal antibodies to *T. gondii*, the protozoan parasite involved in toxoplasmosis have been developed (Kasper *et al.*, *J. Immunol.* 129: 1694-1699 (1982)). MAbs have been developed against schistosomular surface antigens and have been found to act against schistosomulae *in vivo* or *in vitro* (Simpson *et al.*, *Parasitology* 83: 163-177 (1981); Smith *et al.*, *Parasitology* 84: 83-91 (1982); Gryzch *et al.*, *J. Immunol.* 129: 2739-2743 (1982); Zodda *et al.*, *J. Immunol.* 129: 2326-2328 (1982); Dissous *et al.*, *J. Immunol.* 129: 2232-2234 (1982)).

- It should be noted that mixtures of antibodies and immunoglobulin classes can be used, as can hybrid antibodies. Multispecific, including bispecific and hybrid, antibodies and antibody fragments are especially preferred in the methods of the present invention for detecting and treating target tissue and are comprised of at least two different substantially monospecific antibodies or antibody fragments, wherein at least two of said antibodies or antibody fragments specifically bind to at least two different antigens produced or associated with the targeted lesion or at least two different epitopes or molecules of a marker substance produced or associated with the target tissue. Multispecific antibodies and antibody fragments with dual specificities can be prepared analogously to the anti-tumor marker hybrids disclosed in U.S. Pat. No. 4,361,544. Other techniques for preparing hybrid antibodies are disclosed in, e.g., U.S. Pat. Nos. 4,474,893 and 4,479,895, and in Milstein *et al.*, *Immunol. Today* 5: 299 (1984).

- Antibody fragments useful in the present invention include F(ab')₂, F(ab)₂, Fab', Fab, Fv and the like including hybrid fragments. Preferred fragments are Fab', F(ab')₂, Fab, and F(ab)₂. Also useful are any

subfragments retaining the hypervariable, antigen-binding region of an immunoglobulin and having a size similar to or smaller than a Fab' fragment. This will include genetically engineered and/or recombinant proteins, whether single-chain or multiple-chain, which incorporate an antigen-binding site and otherwise function in vivo as targeting vehicles in substantially the same way as natural immunoglobulin fragments. Such single-chain binding molecules are disclosed in U.S. Pat. No. 4,946,778, which is hereby incorporated by reference. Fab' antibody fragments may be conveniently made by reductive cleavage of F(ab')₂ fragments, which themselves may be made by pepsin digestion of intact immunoglobulin. Fab antibody fragments may be made by papain digestion of intact immunoglobulin, under reducing conditions, or by cleavage of F(ab)₂ fragments which result from careful papain digestion of whole immunoglobulin.

A ligand or one member of a ligand-receptor binding pair can be conjugated to the compounds provided herein for targeting the compounds to specific target tissues or target compositions. Examples of ligand-receptor binding pairs are set out in U.S. Pat. Nos. 4,374,925 and 3,817,837, the teachings of which are incorporated herein by reference.

c. Conjugation to Ligands

Many compounds that can serve as targets for ligand-receptor binding pairs, and more specifically, antibodies, have been identified, and the techniques to construct conjugates of such ligands with photosensitizers are well known to those of ordinary skill in this art. For example, Rakestraw *et al.* teaches conjugating Sn(IV) chlorin e via covalent bonds to monoclonal antibodies using a modified dextran carrier (Rakestraw, S. L., Tompkins, R. D., and Yarmush, M. L., *Proc. Natl. Acad. Sci. USA* 87: 4217-4221 (1990)). The compounds disclosed herein can also be conjugated to a ligand, such as an antibody, by using a coupling agent. Any bond which is capable of linking the components such that they are stable under physiological conditions for the time needed for administration and treatment is suitable, but covalent linkages are preferred. The link between two components may be direct, e.g., where a photosensitizer is linked directly to a targeting agent, or indirect, e.g., where a

photosensitizer is linked to an intermediate and that intermediate being linked to the targeting agent.

A coupling agent should function under conditions of temperature, pH, salt, solvent system, and other reactants that substantially retain the chemical stability of the photosensitizer, the backbone (if present), and the targeting agent. Coupling agents should link component moieties stably, but such that there is only minimal or no denaturation or deactivation of the photosensitizer or the targeting agent. Many coupling agents react with an amine and a carboxylate, to form an amide, or an alcohol and a carboxylate to form an ester. Coupling agents are known in the art (see, e.g., M. Bodansky, "Principles of Peptide Synthesis", 2nd ed., and T. Greene and P. Wuts, "Protective Groups in Organic Synthesis," 2nd Ed, 1991, John Wiley, NY).

The conjugates of the compounds provided herein with ligands such as antibodies can be prepared by coupling the compound to targeting moieties by cleaving the ester on the "d" ring and coupling the compound via peptide linkages to the antibody through an N terminus, or by other methods known in the art. A variety of coupling agents, including cross-linking agents, can be used for covalent conjugation. Examples of cross-linking agents include N,N'-dicyclohexylcarbodiimide (DCC), N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), ortho-phenylene-dimaleimide (o-PDM), and sulfosuccinimidyl 4-(N-maleimido-methyl)-cyclohexane-1-carboxylate (sulfo-SMCC). See, e.g., Karpovsky *et al. J. Exp. Med.* 160:1686 (1984); and Liu, MA *et al., Proc. Natl. Acad. Sci. USA* 82: 8648 (1985). Other methods include those described by Brennan *et al. Science* 229: 81-83 (1985) and Glennie *et al., J. Immunol.* 139: 2367-2375 (1987). A large number of coupling agents for peptides and proteins, along with buffers, solvents, and methods of use, are described in the Pierce Chemical Co. catalog, pages O-90 to O-110 (1995, Pierce Chemical Co., 3747 N. Meridian Rd., Rockford Ill., 61105, U.S.A.), which catalog is hereby incorporated by reference.

For example, DCC is a useful coupling agent that can be used to promote coupling of the alcohol NHS to chlorin e6 in DMSO forming an

activated ester which can be cross-linked to polylysine. DCC is a carboxy-reactive cross-linker commonly used as a coupling agent in peptide synthesis, and has a molecular weight of 206.32. Another useful cross-linking agent is SPDP, a heterobifunctional cross-linker for use with primary amines and
5 sulfhydryl groups. SPDP has a molecular weight of 312.4, a spacer arm length of 6.8 angstroms, is reactive to NHS-esters and pyridyldithio groups, and produces cleavable cross-linking such that, upon further reaction, the agent is eliminated so the photosensitizer can be linked directly to a backbone or targeting agent. Other useful conjugating agents are SATA for introduction
10 of blocked SH groups for two-step cross-linking, which is deblocked with hydroxylamine-HCl, and sulfo-SMCC, reactive towards amines and sulfhydryls. Other cross-linking and coupling agents are also available from Pierce Chemical Co. Additional compounds and processes, particularly those involving a Schiff base as an intermediate, for conjugation of proteins to other
15 proteins or to other compositions, for example to reporter groups or to chelators for metal ion labeling of a protein, are disclosed in EPO 243,929 A2 (published Nov. 4, 1987).

Photosensitizers which contain carboxyl groups can be joined to lysine ϵ -amino groups in the target polypeptides either by preformed reactive esters
20 (such as N-hydroxy succinimide ester) or esters conjugated *in situ* by a carbodiimide-mediated reaction. The same applies to photosensitizers which contain sulfonic acid groups, which can be transformed to sulfonyl chlorides which react with amino groups. Photosensitizers which have carboxyl groups can be joined to amino groups on the polypeptide by an in situ carbodiimide
25 method. Photosensitizers can also be attached to hydroxyl groups, of serine or threonine residues or to sulfhydryl groups of cysteine residues.

Methods of joining components of a conjugate, e.g., coupling polyamino acid chains bearing photosensitizers to antibacterial polypeptides, can use heterobifunctional cross linking reagents. These agents bind a functional
30 group in one chain and to a different functional group in the second chain. These functional groups typically are amino, carboxyl, sulfhydryl, and aldehyde. There are many permutations of appropriate moieties which will

react with these groups and with differently formulated structures, to conjugate them together. See the Pierce Catalog, and Merrifield, R. B. *et al.*, *Ciba Found Symp.* 186: 5-20 (1994).

8. Articles of manufacture

- 5 The compounds or pharmaceutically acceptable derivatives thereof may be packaged as articles of manufacture containing packaging material, a compound or pharmaceutically acceptable derivative thereof provided herein, which is effective for modulating the activity of hyperproliferating tissue or neovascularization, or for treatment, prevention or amelioration of one or more
- 10 symptoms of hyperproliferating tissue or neovascularization mediated diseases or disorders, or diseases or disorders in which hyperproliferating tissue or neovascularization activity, is implicated, within the packaging material, and a label that indicates that the compound or composition, or pharmaceutically acceptable derivative thereof, is used for modulating the
- 15 activity of hyperproliferating tissue or neovascularization, or for treatment, prevention or amelioration of one or more symptoms of hyperproliferating tissue or neovascularization mediated diseases or disorders, or diseases or disorders in which hyperproliferating tissue or neovascularization is implicated.

- The articles of manufacture provided herein contain packaging
- 20 materials. Packaging materials for use in packaging pharmaceutical products are well known to those of skill in the art. See, *e.g.*, U.S. Patent Nos. 5,323,907; 5,052,558 and 5,033,252. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material
- 25 suitable for a selected formulation and intended mode of administration and treatment. A wide array of formulations of the compounds and compositions provided herein are contemplated as are a variety of treatments for any disease or disorder in which hyperproliferating tissue or neovascularization is implicated as a mediator or contributor to the symptoms or cause.

30

E. METHODS OF USE OF THE COMPOUNDS AND COMPOSITIONS

1. Methods of PDT, Diagnostic and Therapeutic Applications

Briefly, the photosensitizing compound is generally administered to the subject before the target tissue, target composition or subject is subjected to
5 illumination. The photosensitizing compound is administered as described elsewhere herein.

The dose of photosensitizing compound can be determined clinically. Depending on the photosensitizing compound used, an equivalent optimal therapeutic level will have to be established. A certain length of time is
10 allowed to pass for the circulating or locally delivered photosensitizer to be taken up by the target tissue. The unbound photosensitizer is cleared from the circulation during this waiting period, or additional time can optionally be provided for clearing of the unbound compound from non-target tissue. The waiting period will be determined clinically and may vary from compound to
15 compound.

At the conclusion of this waiting period, a laser light source or a non-laser light source is used to activate the bound drug. The area of illumination is determined by the location and dimension of the pathologic region to be detected, diagnosed or treated. The duration of illumination period will depend
20 on whether detection or treatment is being performed, and can be determined empirically. A total or cumulative period of time anywhere from between about 4 minutes and 72 hours can be used. In one embodiment, the illumination period is between about 60 minutes and 148 hours. In another embodiment, the illumination period is between about 2 hours and 24 hours.

25 Preferably, the total fluence or energy of the light used for irradiating, as measured in Joules, is between about 10 Joules and about 25,000 Joules; more preferably, between about 100 Joules and about 20,000 Joules; and most preferably, between about 500 Joules and about 10,000 Joules. Light of a wavelength and fluence sufficient to produce the desired effect is selected,
30 whether for detection by fluorescence or for therapeutic treatment to destroy or impair a target tissue or target composition. Light having a wavelength corresponding at least in part with the characteristic light absorption

wavelength of the photosensitizing agent is preferably used for irradiating the target issue.

The intensity or power of the light used is measured in watts, with each Joule equal to one watt-sec. Therefore, the intensity of the light used for
5 irradiating in the present invention may be substantially less than 500 mW/cm². Since the total fluence or amount of energy of the light in Joules is divided by the duration of total exposure time in seconds, the longer the amount of time the target is exposed to the irradiation, the greater the amount of total energy or fluence may be used without increasing the amount of the
10 intensity of the light used. The present invention employs an amount of total fluence of irradiation that is sufficiently high to activate the photosensitizing agent.

While not wishing to be limited by a theory, the inventor proposes that the disclosed photosensitizing compounds can be substantially and selectively
15 photoactivated in target tissue or target compositions within a therapeutically reasonable period of time and without excess toxicity or collateral damage to non-target tissues.

In one embodiment of using compounds disclosed herein for photodynamic therapy, the compounds are injected into the mammal, *e.g.*
20 human, to be diagnosed or treated. The level of injection is usually between about 0.1 and about 0.5 μ mol/kg of body weight. In the case of treatment, the area to be treated is exposed to light at the desired wavelength and energy, *e.g.* from about 10 to 200 J/cm². In the case of detection, fluorescence is determined upon exposure to light at a wavelength sufficient to cause the
25 compound to fluoresce at a wavelength different than that used to illuminate the compound. The energy used in detection is sufficient to cause fluorescence and is usually significantly lower than is required for treatment.

Any one of the photosensitizing compounds disclosed herein or a pharmaceutically acceptable derivative thereof may be supplied in a kit along
30 with instructions on conducting any of the methods disclosed herein. Instructions may be in any tangible form, such as printed paper, a computer disk that instructs a person how to conduct the method, a video cassette

containing instructions on how to conduct the method, or computer memory that receives data from a remote location and illustrates or otherwise provides the instructions to a person (such as over the Internet). A person may be instructed in how to use the kit using any of the instructions above or by

5 receiving instructions in a classroom or in the course of treating a patient using any of the methods disclosed herein, for example.

2. Imaging Enhancing Agents

In addition to PDT, the compositions provided herein can be used as imaging enhancing agents in diagnostic imaging techniques, or for the labeling

10 of target tissues or target compositions for diagnostic radiology. In the modern medical field, there are a variety of treatments including magnetic resonance imaging (MRI) for the diagnosis of diseases. Detection of cancer in its early stages should improve the ability to cure eliminate the cancerous tissue. Early diagnosis of precancerous regions and minute cancer are

15 important subject matters in modern cancer treatments. MRI has emerged as a powerful tool in clinical settings because it is noninvasive and yields an accurate volume rendering of the subject. The image is created by imposing one or more orthogonal magnetic field gradients upon the subject or specimen while exciting nuclear spins with radio frequency pulses as in a typical nuclear

20 magnetic resonance (NMR) experiment. After collection of data with a variety of gradient fields, deconvolution yields a one, two, or three dimensional image of the specimen/subject. Typically, the image is based on the NMR signal from the protons of water where the signal intensity in a given volume element is a function of the water concentration and relaxation times. Local variation in

25 there parameters provide the vivid contrast observed in MR images.

MRI contrast agents act by increasing the rate of relaxation, thereby increasing the contrast between water molecules in the region where the imaging agent accretes and water molecules elsewhere in the body. However, the effect of the agent is to decrease both T_1 and T_2 , the former resulting in

30 greater contrast while the latter results in lesser contrast. Accordingly, the phenomenon is concentration-dependent, and there is normally an optimum

concentration of a paramagnetic species for maximum efficacy. This optimal concentration will vary with the particular agent used, the locus of imaging, the mode of imaging, *i.e.*, spin-echo, saturation-recovery, inversion-recovery and/or various other strongly T_1 -dependent or T_2 -dependent imaging

5 techniques, and the composition of the medium in which the agent is dissolved or suspended. These factors, and their relative importance are known in the art. See, *e.g.*, Pykett, *Scientific American* 246: 78 (1982); Runge *et al.*, *Am. J. Radiol.* 141: 1209 (1983). When MRI contrast agents are used diagnostically, they are vascularly perfused, enhancing the contrast of blood vessels and

10 reporting on organ lesions and infiltration. However, the labeling of specific tissues for diagnostic radiology remains a difficult challenge for MRI. Efforts to develop cell and tissue-specific MRI image enhancing agents by modifying existing immunological techniques has been the focus of much research in diagnostic radiology. For example, antibodies labeled with paramagnetic ions,

15 generally the gadolinium chelate Gd-DTPA, have been generated and tested for their effects on MRI contrast of tumors and other tissues (U.S. Pat. No. 5,059,415). Unfortunately, the relaxivity of Gd bound to antibodies has been found to be only slightly better than that of unbound Gd-DTPA (Paajanen *et al.*, *Magn. Reson. Med* 13: 38-43 (1990)).

20 MRI is generally used to detect ^1H nuclei in the living body. However, MRI is capable of detecting NMR spectrums of other nuclear species, including ^{13}C , ^{15}N , ^{31}P , and ^{19}F . The ^{19}F is not abundant in the living body. By incorporating isotopes useful in MRI, such as ^{13}C , ^{15}N , ^{31}P , or ^{19}F , and particularly ^{19}F in the compositions provided herein and administering to a

25 subject, the compounds provided herein would accumulate in target tissue, and subsequent MR imaging would produce NMR data with enhanced signal from the targeted tissue or target compositions due to the presence of the accumulated compound with the MRI recognizable isotope, such as ^{19}F . Thus, the disclosed compounds can be used as image enhancing agents and will

30 provide labeling of specific target tissues or target compositions for diagnostic radiology, including MRI.

3. Detecting Target Tissue or Target Compositions

In addition to PDT, the compositions provided herein can be used to detect target cells, target tissue, or target compositions in a subject. When the compounds provided herein are to be used for detection of target tissue or target composition, the compounds are introduced into the subject and sufficient time is allowed for the compounds to accumulate in the target tissue or to become associated with the target composition. The area of treatment is then irradiated, generally using light of an energy sufficient to cause fluorescence of the compound, and the energy used is usually significantly lower than is required for photodynamic therapy treatment. Fluorescence is determined upon exposure to light at the desired wavelength, and the amount of fluorescence can be correlated to the presence of the compound, qualitatively or quantitatively, by methods known in the art.

4. Diagnosing an Infecting Agent

The compositions provided herein can be used to diagnose the presence of an infecting agent, or the identity of an infecting agent in a subject. The compounds provided herein can be conjugated to one or more ligands specific for an infecting agent, such as an antibody or antibody fragment, that selectively associates with the infecting agent, and after allowing sufficient time for the targeted compound to associate with the infecting agent and to clear from non-target tissue, the compound can be visualized, such as by exposing to light of an energy sufficient to cause fluorescence of the compound, or by imaging using diagnostic radiology, including MRI. By way of example, any one of the compounds provided herein can be conjugated to an antibody that is targeted against a suitable *Helicobacter pylori* antigen, and formulated into a pharmaceutical preparation that, when introduced into a subject, releases the conjugated compound to a gastric mucus/epithelial layer where the bacterium is found. After sufficient time for the compound to selectively associate with the target infecting agent, and for any unbound compound to clear from non-target tissue, the subject can be examined to determine whether any *Helicobacter pylori* is present. This can be done by MRI to detect accumulated compound because of the presence of ^{19}F substituents,

for example, or by irradiating the suspect target area with light of an energy sufficient to cause fluorescence of the compound, such as by using fiberoptics, and detecting any fluorescence of the targeted compound.

F. ASSAY METHODS USED FOR TESTING THE COMPOUNDS

- 5 The compounds provided herein have been evaluated in *in vivo* studies in a mouse tumor model system (RIF tumor). The compounds were tested at variable concentrations by incubating with RIF cells, and after a 3 hour incubation in the dark at 37°C, the cells were washed with PBS, then irradiated with a 1000W Quartz Halogen Lamp with IR and bandpass dichroic filters to
- 10 allow light between 400 nm - 700 nm, at a dose rate of 16 mW/cm² at 700 nm. The percent cell kill rate was then determined for each condition.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the subject matter claimed herein.

EXAMPLES

- 15 Melting points are uncorrected and were measured on a Fisher Johns melting point apparatus. Electronic absorption spectra were measured on a Genesis 5 spectrophotometer. Analytical thin layer chromatography was used to monitor the reactions and to check the purity of the desired compounds on cut strips of Merck or Whatman silica gel 60F264 precoated (.25 mm
- 20 thickness) plastic backed sheets. For column chromatography Silica gel (70-230 mesh) was used for normal gravity column.

- NMR spectra were recorded in CDCl₃ or TFA solutions at 400 or 600 MHz Bruker instruments. Chemical shifts are expressed in ppm with residual CHCl₃ in CDCl₃ as internal standard (for ¹H, 7.26 ppm). UV-vis spectra were
- 25 recorded on a Varian (Cary-50 Bio) spectrophotometer. Separations on column chromatography were performed on silica gel 60 (70-230 mesh) or neutral alumina (Brockmann grade III, 150 mesh). Preparative TLC was performed on silica 20 x 20 cm TLC plates (Aldrich).

- Before *in vitro* and *in vivo* testing of the compounds, the purity of the
- 30 material was confirmed by HPLC and it was performed using a Spectra-

Physics system connected to a SP8 700 solvent delivery system, Kratos 757 absorption detector with a fixed wavelength ant 405 or 786 nm. Two solvent systems were used in the HPLC analysis: solvent A was prepared by dissolving anhydrous dibasic sodium phosphate (1.0 g) in 400 ml water. To
 5 this was added HPLC grade methanol (60 ml). The pH of the solution was adjusted to 7.5 with phosphoric acid; and (ii) solvent B was prepared by dissolving anhydrous dibasic sodium phosphate (0.3 g) in 100 ml water, and_ to this was added methanol (900 ml) and the pH was adjusted to 7.5 with phosphoric acid. Solvents A and B were used as gradient mode (0 - 10 min A,
 10 10 - 40 min A - B, 40 - 50 min B, 50 - 60 min back to A). In some cases solvent B was used as isocratic mode (column reverse phase C-8, flow rate 1.5 ml/min).

EXAMPLE 1

Preparation of 3-acetyl-bacteriopurpurin-18-methyl ester

15 Bacteriopurpurin-18 methyl-ester 13 was obtained from bacteriochlorophyll-a, which can be isolated from *R. spheroides* or *R. capsulata* (see U.S. Patent 5,591,847). *R. spheroides* (350 gram) was dissolved in ether (200 mL) and pyridine (10 mL). Sodium hydroxide (12g) dissolved in methanol (100 mL) was added and a stream of air was bubbled
 20 through the solution with constant stirring for 2 hours. The ether layer was removed, and the pH of the aqueous phase was adjusted by adding H₂SO₄ to 2.5. The solvent was removed under vacuum. The residue so obtained was redissolved in THF and evaporated. This process was repeated several times until the peak at 765 nm disappeared and a new peak appeared at 804 nm.
 25 After removing the solvent, the residue was found to be a mixture of two compounds and separated by column chromatography. The faster moving band was identified as the title product, whereas the slowing moving band was characterized as the related carboxylic acid analog, which on treating with 5% sulfuric acid/methanol or diazomethane produced the corresponding methyl
 30 ester. Yield: 250 mg. M. P. 272 °C. (Y. Chen, Ph. D. thesis, entitled "Long Wavelength Absorbing Photosensitizers Related to Bacteriochlorins," RCPI/SUNY, Buffalo, September 1, 2002).

EXAMPLE 2

General method for the preparation of N-substituted purpurinimides:

Purpurin-18 methyl ester was prepared by following the literature procedure (Zheng, G.; Potter, W. R.; Camacho, S. H.; Missert, J. R.; Wang, G., Bellnier, D. A., Henderson, B. W., Rodgers, M. A. J., Dougherty, T. J., and Pandey, R. K., *J. Med. Chem.* 44: 1540-1559 (2001)). In a typical experiment, purpurin-18 methyl ester (500 mg) was dissolved in toluene (30 ml) at reflux. A large excess of 3, 5-bis(trimethyl)- or 3, 5-bis(trifluoromethyl)benzylamine was added and the reaction was continued until it was complete. The reaction was monitored spectrophotometrically, and the disappearance of the 699 nm band and the appearance of a new peak at 705 nm in the UV-Vis spectrum indicated the completion of the reaction. The reaction solvent and the excess of the amine were then evaporated under high vacuum to give the residue, which was purified by chromatography on a silica column eluting with 2% acetone in dichloromethane. Evaporation of the solvents from the appropriate fractions afforded the corresponding N-substituted purpurinimide in 70-75% yield. The purpurin-18-N-butylimide was prepared by following the method reported previously (Zheng, G.; Potter, W.R.; Camacho, S.H.; Missert, J.R.; Wang, G.; Bellnier, D.A.; Henderson, B.W.; Rodgers, M.A.J.; Dougherty, T.J.; and Pandey, R.K., *J. Med. Chem.* 44:1540-1559 (2001)).

EXAMPLE 3

General method for the preparation of O-alkyl or O-benzyl (fluorinated or non-fluorinated) N-alkyl or N-aryl-purpurinimides:

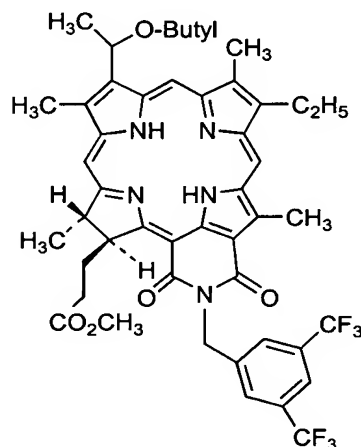
For the preparation of the desired ether analogs, purpurin-18-N-substitute imide (100 mg) was reacted with 30% HBr/AcOH (1.5 mL) in a closed flask using a rubber septum and the reaction mixture was stirred at room temperature for 2 hours. After evaporating the acid under high vacuum and low temperature (around 30°C), the residue was allowed to react with an excess of the desired alcohol. Dry dichloromethane (10 mL) and anhydrous potassium carbonate (50 mg) were then added and the reaction mixture was stirred under the nitrogen atmosphere for 45 min. It was then diluted with

dichloromethane (200 mL) and treated with ethereal diazomethane for 5 min. Before evaporating the solvent, the excess diazomethane was removed by passing a stream of nitrogen through the solution. The residue so obtained

- 5 dichloromethane. The appropriate fractions were combined. Evaporation of the solvent gave the desired compounds in 70-75% yield.

The following compounds were prepared according to the methods of EXAMPLES 2 and 3 by routine modification using appropriate starting materials and reagents.

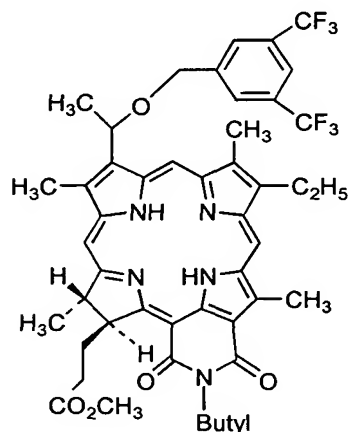
10 **Compound 9**



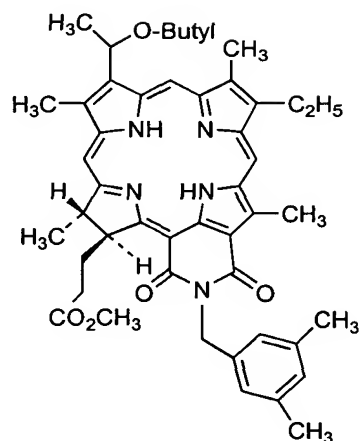
- Melting point: sticky solid. UV-Vis (THF): 700.0 (4.50×10^4), 644.1 (6.71×10^3), 544.0 (1.90×10^4), 508.0 (6.02×10^3), 480.0 (3.54×10^3), 415.0 (1.11×10^5), 364.0 (3.96×10^4). ¹H NMR (400 MHz, CDCl₃) δ , ppm: 9.75 and 9.63 (each s, 1H, for 10H and 5H); 8.52 (s, 1H, for 20H); 8.25 (s, 2H, 2 \times ArH); 7.81 (s, 1H, ArH); 5.77 (s, 3H, N-CH₂ & 3¹CH); 5.34 (d, J = 7.6 Hz, 1H, 17H); 4.35 (q, J = 7.2 Hz, 1H, 18H); 3.84 (s, 3H, 12CH₃); 3.69 (d, J = 6.8 Hz, 2H, 8¹CH₂); 3.55 (s, 3H, OCH₃); 3.31 (s, 3H, 7CH₃); 3.19 (s, 3H, 2CH₃); 2.68 (t, J = 10 Hz, 1H, 1 \times 17¹CH₂CH₂CO₂CH₃); 2.37 (q, J = 10 Hz, 2H, 17²CH₂CH₂CO₂CH₃); 2.06 (d, 3H, 18CH₃); 1.96 (q, J = 9.3 Hz, 1H, 1 \times 17¹CH₂CH₂CO₂CH₃); 1.76 (d, J = 6.8 Hz, 2H, OCH₂); 1.69 (t, J = 7.4 Hz, 3H, 8²CH₃); 1.65 (m, 4H, O-CH₂CH₂CH₂CH₃); 0.89 (t, 3H, -O(CH₂)₃CH₃). 0.15 and 0.90 (each s, 1H 2NH).
- 15
- 20

^{19}F NMR (400 MHz, CDCl_3) δ , ppm: 13.054 (referenced to TFA). Mass calculated for $\text{C}_{47}\text{H}_{49}\text{N}_5\text{O}_5\text{F}_6$: 877.36. Found: 878.3 (M + 1).

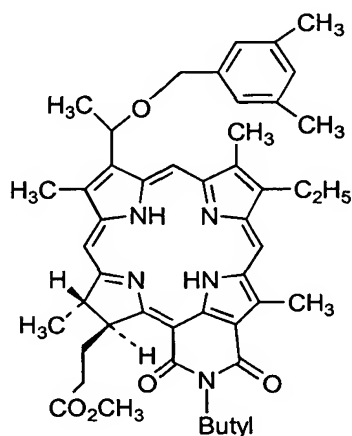
Compound 12



- 5 Melting point: sticky solid. UV-Vis (THF): 700.0 (4.67×10^4), 642.0 (5.97×10^3), 542.1 (1.72×10^4), 507.0 (6.29×10^3), 478.0 (3.40×10^3), 415.0 (1.25×10^5), 364.0 (4.14×10^4). ^1H NMR (400 MHz, CDCl_3) δ , ppm: 9.73 and 9.72 (each s, 1H, for 10H and 5H); 8.60 (s, 1H, for 20H); 7.85 (s, 2H, 2x ArH); 7.81 (s, 1H, ArH); 5.96 (q, J = 6.8 Hz, 1H, 3^1CH); 5.43 (d, J = 8.0 Hz, 1H, 17H);
- 10 4.79 (s, 2H, O- CH_2); 4.48 (t, J = 7.8 Hz, 2H, N- CH_2); 4.38 (q, J = 7.2 Hz, 1H, 18H); 3.86 (s, 3H, 12 CH_3); 3.69 (q, J = 7.6 Hz, 2H, 8^1CH_2); 3.57 (s, 3H, O CH_3); 3.10 (s, 3H, 7 CH_3); 2.68 (s, 1H, 1x $17^1\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.44 (s, 1H, 1x $17^1\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.32 (s, 1x $17^2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.21 (d, J = 6.4 Hz, 3H, 3^2CH_3); 1.99 (m, J = 5.4 Hz, 2H, N- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ & 17^2H); 1.78 (d, J = 6.8
- 15 Hz, 3H, 18 CH_3); 1.66 (m, J = 7.9 Hz, 2H, N- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ & 3H, 8^2CH_3); 1.11 (t, J = 7.4 Hz, 3H, N- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); -0.172 and 0.17 (each s, 1H, 2NH). ^{19}F NMR (400 MHz, CDCl_3) δ , ppm: 12.598 (referenced to TFA). Mass calculated for $\text{C}_{47}\text{H}_{49}\text{N}_5\text{O}_5\text{F}_6$: 877.36. Found: 879 (M+2).

Compound 8

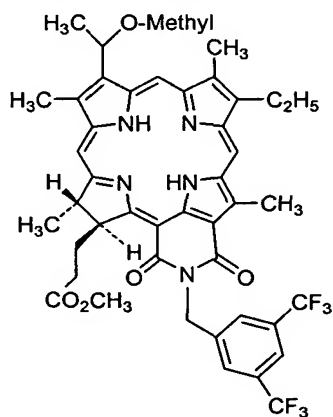
Melting point: sticky solid. UV-Vis (THF): 697.9 (4.96×10^4), 640.9 (9.15×10^3), 542.1 (2.11×10^4), 506.0 (1.01×10^4), 415.0 (1.33×10^5), 363.0 (4.59×10^4). ¹H NMR (400 MHz, CDCl₃) δ , ppm: 9.75 and 9.64 (each s, 1H, for 10H and 5H); 8.53 (s, 1H, for 20H); 7.33 (s, 2H, 2x ArH); 6.89 (s, 1H, ArH); 5.78 (q, J = 6.8 Hz, 1H, 3¹CH); 5.62 (q, J = 12.8, 2H, OCH₂); 5.42 (d, J = 7.6 Hz, 1H, 17H); 4.33 (q, J = 7.2 Hz, 1H, 18H); 3.83 (s, 3H, 12CH₃); 3.53 (s, 3H, 17²CH₂CH₂CO₂CH₃); 3.30 (s, 3H, 7CH₃); 3.18 (s, 3H, 2 CH₃); 2.31 (s, 6H, 2x ArH-CH₃); 2.05 (d, J = 6.6 Hz, 2H, 8²CH₃); 1.75 (d, J = 6.8 Hz, 3H, 18CH₃); 1.52-1.67 (m, 4H, -O-CH₂CH₂CH₂CH₃); 0.862 (t, 3H, O-CH₂CH₂CH₂CH₃); -0.046 (s, 1H, NH); -0.153 (s, 1H, NH). Mass calculated for C₄₇H₅₅N₅O₅: 769.42. Found: 792.4 (M + Na).

Compound 11

15

- Melting point: sticky solid. UV-Vis (THF): 699.0 (4.44×10^4), 642.0 (6.32×10^3), 543.1 (1.70×10^4), 508.1 (6.54×10^3), 478.0 (3.76×10^3), 415.0 (1.23×10^5), 363.9 (4.04×10^4). ^1H NMR (400 MHz, CDCl_3) δ , ppm: 9.79 and 9.63 (each s, 1H, for 10H and 5H); 8.60 (s, 1H, for 20H); 7.79 (s, 1H, ArH); 6.96 (m, 2H, 2x ArH); 5.91 (q, 1H, 3^1CH); 5.44 (m, 1H, 17H); 4.75 (2H, OCH_2); 4.49 (t, $J = 7.8$ Hz, 2H, N-CH_2); 4.39 (m, 1H, 18H); 3.82 (s, 3H, 12CH_3); 3.66 (q, $J = 7.6$ Hz, 2H, 8^1CH_2); 3.58 (s, 3H, $17^2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 3.34 (m/s, 3H, 7CH_3); 3.12 (s, 3H, 2CH_3); 2.70 (m, 1H, $17^1\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.46 (m, 1H, $17^2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.25 (s, 6H, 2x ArH- CH_3); 2.12 (d, $J = 6.4$ Hz, 3H, 8^2CH_3); 1.70 (d, 3H, 18CH_3); 1.62-1.70 (m, 6H, $\text{N-CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); 1.12 (t, $J = 3.52$ Hz, 3H, $\text{N-CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); -0.106 and -0.12 (each s, 1H, 2NH). Mass calculated for $\text{C}_{47}\text{H}_{55}\text{N}_5\text{O}_5$: 769.42. Found: 770 ($\text{M}+1$).

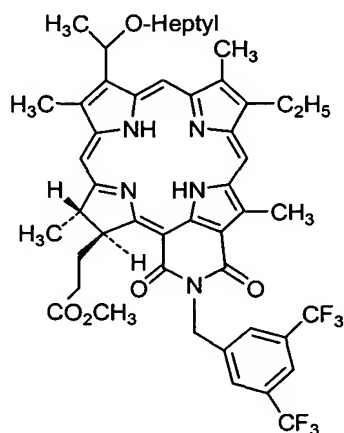
Compound 9b ($\text{R}^1 = \text{methyl}$)



- 15 Melting point: sticky solid. UV-Vis (THF): 700.0 (4.41×10^4), 646.1 (6.05×10^3), 544.0 (1.82×10^4), 506.9 (5.42×10^3), 415.0 (1.21×10^5), 364.0 (3.93×10^4). ^1H NMR (400 MHz, CDCl_3) δ , ppm: 9.65 and 9.46 (each s, 1H, for 10H and 5H); 8.56 (s, 1H, for 20H); 8.24 (s, 2H, 2x ArH); 7.82 (s, 1H, ArH); 5.74 (s, 3H, N-CH_2 & 3^1CH); 5.36 (d, $J = 7.1$ Hz, 1H, 17H); 4.37 (q, $J = 7.3$ Hz, 1H, 18H); 3.68 (s, 3H, 12CH_3); 3.69 (d, $J = 6.8$ Hz, 2H, 8^1CH_2); 3.55 (s, 6H, OCH_3 merged with a peripheral CH_3); 3.33 (s, 3H, 7CH_3); 3.16 (s, 3H, 2CH_3); 2.71 (t, $J = 9.9$ Hz, 1H, 1x $17^1\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.39 (q, $J = 9.7$ Hz, 2H, $17^2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 2.07 (m, 3H, 8^2CH_3); 1.97 (q, $J = 9.6$ Hz, 1H, 1x $17^1\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$); 1.80 (d, $J = 7.6$ Hz, 3H, 18CH_3); 1.62 (t, $J = 7.8$ Hz, 3H,

8²CH₃); 0.06 and 0.02 (each s, 1H, 2NH). ¹⁹F NMR (400 MHz, CDCl₃) δ, ppm: 13.284 (referenced to TFA). Mass calculated for C₄₄H₄₃N₅O₅F₆: 835.32. Found: 858.5 (M + Na).

Compound 9c (R¹ = heptyl)



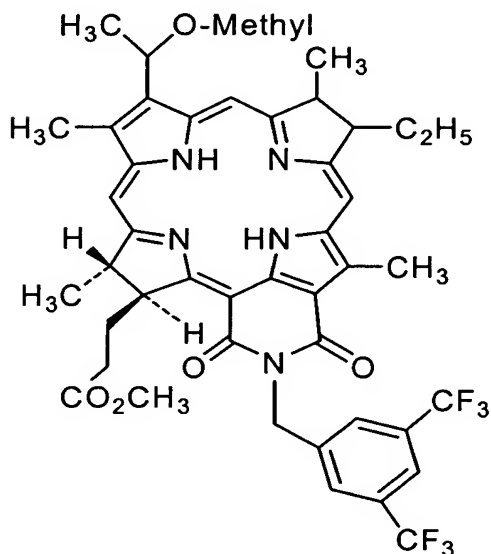
5

Melting point: sticky solid. UV-Vis (THF): 700.0 (4.56×10^4), 644.1 (5.92×10^3), 544.9 (1.83×10^4), 508.0 (4.78×10^3), 416.0 (1.22×10^5), 363.0 (3.86×10^4). ¹H NMR (400 MHz, CDCl₃) δ, ppm: 9.74 and 9.51 (each s, 1H, for 10H and 5H); 8.52 (s, 1H, for 20H); 8.23 (s, 2H, 2x ArH); 7.81 (s, 1H, ArH); 5.74 (s, 3H, N-CH₂ & 3¹CH); 5.35 (d, J = 8.4 Hz, 1H, 17H); 4.35 (q, J = 7.3 Hz, 1H, 18H); 3.73 (s, 3H, 12CH₃); 3.60 (d, J = 4.0 Hz, 2H, 8¹CH₂); 3.54 (s, 3H, OCH₃); 3.30 (s, 3H, 7CH₃); 3.16 (s, 3H, 2CH₃); 2.68 (t, J = 9.7 Hz, 1H, 1x 17¹CH₂CH₂CO₂CH₃); 2.42 (q, J = 13 Hz, 2H, 17²CH₂CH₂CO₂CH₃); 2.11 (m, 3H, 8²CH₃); 2.01 (q, J = 9.8 Hz, 1H, 1x 17¹CH₂CH₂CO₂CH₃); 1.64 (d, 3H, 18CH₃); 1.18-1.50 (several peaks, CH₂'s of heptyl); 0.80 (t, 3H, CH₃ of the hexyl group); 0.20 and 0.15 (each s, 1H, NH). ¹⁹F NMR (400 MHz, CDCl₃) δ, ppm: 13.031 (referenced to TFA). Mass calculated for C₅₀H₅₅N₅O₅F₆: 919.41. Found: 942.4 (M + Na).

10

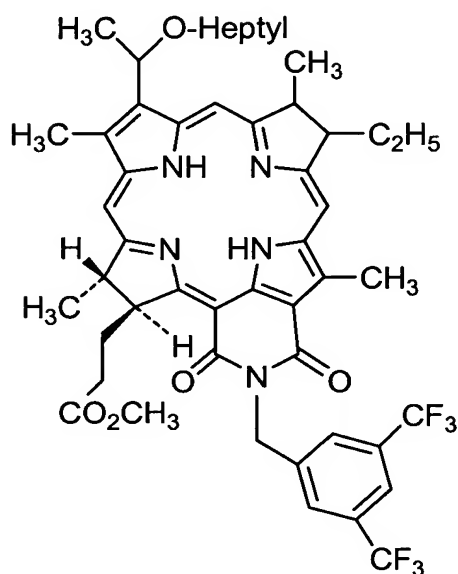
15

Compound 16 (R = methyl)

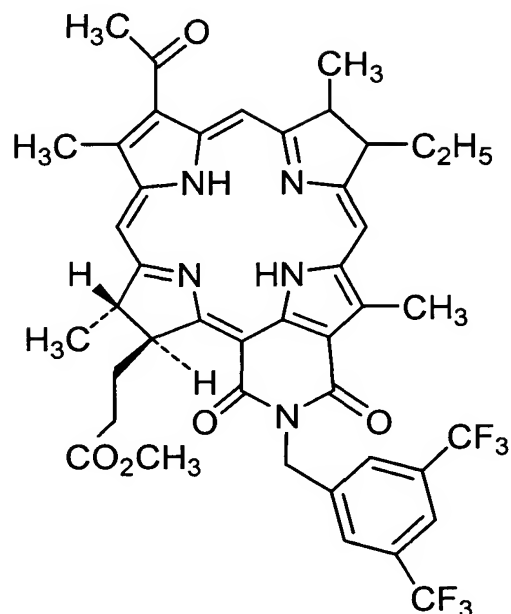


Melting point: sticky solid. UV-Vis (CH₂Cl₂): 784.0 (4.00 × 10⁴), 719.1 (1.30 × 10⁴), 538.0 (3.86 × 10⁴), 503.0 (5.55 × 10³), 417.1 (5.41 × 10⁴), 367.0 (9.99 × 10⁴), 346.0 (4.86 × 10⁴). ¹H NMR (400 MHz, CDCl₃) δ, ppm: 8.75 (splitting, 1H, 5-Meso H); 8.55, 8.30 (each s, 1H, 10, 20- meso H); 8.12 (s, 2H, 2-ArH); 7.75 (s, 1H, Ar-H); 5.65 (s, N-CH₂); 5.20 (m, 1H, 17H); 4.25 (m, 2H, 7H and 18H); 4.00 (m, 1H, 8H); 3.65, 3.55 and 3.50 (each s, 3H, -CO₂CH₃ and 2 × CH₃); 3.02 (s, 3H, -COCH₃); 2.70-1.80 (several m, 17¹H, 17²H, 8¹-CH₂, total 4H); 1.98 (two doublets merged, 6H, 18-CH₃ and 7-CH₃); 1.60 (d, 3H, 3¹CH₃); 1.10 (t, 3H, 7²-CH₃); 0.35 and -0.10 (each s, 1H, 2NH). ¹⁹F NMR (400 MHz, CDCl₃) δ, ppm: 13.136 (referenced to TFA). Mass calculated for C₅₀H₅₇N₅O₅F₆: 837.33. Found: 860.4 (M + Na).

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Compound 16 (R = heptyl)

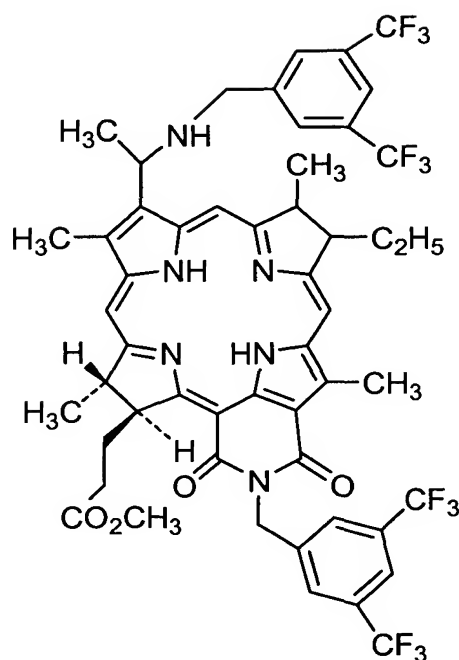
Melting point: sticky solid. UV-Vis (CH_2Cl_2): 784.0 (4.06×10^4), 730.0 (1.26×10^4), 537.0 (3.90×10^4), 505.0 (5.50×10^3), 472.0 (5.24×10^3), 417.1 (4.60×10^4), 367.0 (9.80×10^4), 346.9 (4.68×10^4). ^1H NMR (400 MHz, CDCl_3) δ , ppm: 8.80 and 8.78 (splitting, 1H, 5-Meso H); 8.56, 8.27 (each s, 1H, 10, 20-meso H); 8.16 (s, 2H, 2-ArH); 7.82 (s, 1H, Ar-H); 5.65 (s, 2H, N- CH_2); 5.70 (q, 1H, 3¹ CH); 5.20 (m, 1H, 17-H); 4.25 (m, 2H, 7H and 18H); 4.00 (m, 1H, 8-H); 3.65, 3.55 and 3.20 (each s, 3H, 17- $\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$, 2- CH_3 , 12- CH_3); 2.70-1.80 (several m, 17¹H, 17²H, 8¹- CH_2 , total 4H); 2.00 and 1.75 (each d, 3H, 18- CH_3 and 7- CH_3); 1.75 (merged d 3H, 3¹ CH_3); 1.20-1.80 (several m, 12H, CH_2 's of heptyl); 1.10 (t, 3H, CH_3 of O-heptyl); 0.45 and -0.05 (each s, 1H, 2NH). ^{19}F NMR (400 MHz, CDCl_3) δ , ppm: 13.137 (referenced to TFA). Mass calculated for $\text{C}_{44}\text{H}_{45}\text{N}_5\text{O}_5\text{F}_6$: 921.43. Found: 944.5 (M + Na).

Compound 15

Melting point: sticky solid. UV-Vis (THF): 822.0 (6.01×10^4), 547.0 (3.63×10^4), 416.0 (4.38×10^4), 365.0 (8.38×10^4). ^1H NMR (400 MHz, CDCl_3) δ ,

5 ppm: 9.20, 8.75 and 8.60 (each s, 1H, meso H); 8.12 (s, 2H, 2-ArH); 7.75 (s, 1H, ArH); 5.75 (s, 2H, N- CH_2); 5.20 (m, 1H, 17H); 4.25 (m, 2H, 7H and 18H); 4.05 (m, 1H, 8H); 3.65 (s, 3H, CH_3); 3.50 (s, 6H, 2 CH_3); 3.02 (s, 3H, COCH_3); 2.70-1.80 (several m, 17 ^1H , 17 ^2H , 8 $^1\text{-CH}_2$, total 4H); 1.80, 1.65 (each d, 3H, 18- CH_3 and 7- CH_3); 1.10 (t, 3H, 7 $^2\text{-CH}_3$); -0.80 and -0.70 (each s, 1H, 2NH).

10 Mass calculated for $\text{C}_{43}\text{H}_{41}\text{N}_5\text{O}_5\text{F}_6$: 821.30. Found: 844.4 (M + Na).

Compound 18

Melting point: sticky solid. UV-Vis (CH₂Cl₂): THF 784.1 (3.83×10^4), 538.0 (3.86×10^4), 470.1 (4.87×10^3), 417.1 (4.61×10^4), 367.9 (9.60×10^4). ¹H

- 5** *NMR* (400 MHz, CDCl₃) δ , ppm: 9.02 and 9.05 [each s, 0.5H (splitting of 5H meso proton)]; 8.50 and 8.25 (each s, 1H, meso H); 8.18 (s, 2H, 2 x Ar H); 7.75 (s, 1H, ArH); 7.65-7.80 (several s, 3H, ArH); 5.12 (m, 2H, 3¹CH and 18H); 4.18 (m, 2H, 17 H and 7H); 4.14 (m, 1H, 8H); 4.00 (m, 1H, -NH-CH₂); 3.55, 3.52 and 3.10 (each s, 3H, -CO₂CH₃, 12-CH₃ and 2-CH₃); 2.70-1.80
- 10** (several m, 17¹H, 17²H, 8¹-CH₂, total 4H); 1.80, 1.65 (each d, 3H, 3¹CH₃, 18-CH₃ and 7-CH₃); 1.10 (t, 3H, 7²-CH₃); 0.40 and -0.20 (each s, 1H, 2NH). Mass calculated for C₅₂H₄₈N₆O₄F₁₂: 1048.35. Found: 1049.3 (M + 1).

- All ether analogs were isolated as diastereomeric mixtures. In the case of the purpurinimide analogs, no significant separation in the resonances of various protons was observed. However, in the bacteriochlorin series, the
- 15** *meso*-proton (position-5) adjacent to the ether side chain (chiral center at position-3) showed two clear singlets for the resonances of both the isomers.

EXAMPLE 4

In Vitro Photosensitizing Efficacy of Chlorin-related Compounds

RIF tumor cells were grown in alpha-DMEM with 10% fetal calf serum, penicillin and streptomycin. Cells were maintained in 5% CO₂, 95% air and 100% humidity. For determining PDT efficacy, these cells were plated in 96-well plates at a density of 1×10^4 cells well in complete media. After overnight incubation, in initial experiments in order to select the optimal dose, the substantially pure fluorinated photosensitizer **9** was initially added at variable concentrations. After a 3 hour incubation in the dark at 37°C, the cells were washed with PBS, then irradiated with a 1000W Quartz Halogen Lamp with IR and bandpass dichroic filters to allow light between 400 nm - 700 nm, at a dose rate of 16 mW/cm² at 700 nm. As can be seen from FIGURE 2, compound **9** at 2.5 μ M concentration produced 100% cell kill on exposing to light at a dose of 4J/cm². The other fluorinated and non-fluorinated purpurinimide analogs were then evaluated under similar conditions. Among the compounds evaluated, the fluorinated analogs produced better photosensitizing activity and the results are summarized in FIGURE 3, which shows the *in vitro* photosensitizing efficacy of fluorinated (**9**, **12**) and non-fluorinated (**8**, **11**) purpurinimide-based photosensitizers at a concentration of 2.5 μ M in RIF tumor cells, where the control is photosensitizer only with no light.

EXAMPLE 5

In Vivo Photosensitizing Activity of Chlorin related compounds

The fluorinated and non-fluorinated photosensitizers **8**, **9**, **11** and **12** were also evaluated for their *in vivo* tumor response in C3H mice transplanted with RIF tumors (Henderson, B. W.; Bellnier, D. A.; Graco, W. R.; Sharma, A.; Pandey, R. K.; Vaughan, L.; Weishaupt, K. R.; and Dougherty, T. J., *Cancer Res.* 57: 4000 (1997)). In brief, C3H/HEJ mice were subjected to intradermal injection with 2×10^5 RIF cells in 30mL HBSS w/o Ca⁺² and magnesium, into the flank and allowed to grow until they were 4-5 mm in diameter. The day before tumors were treatment size, the mice were injected with variable doses

of substantially pure photosensitizers (1.0 mg/kg, 0.4 mg/kg). At 24 hours post injection the mice were anesthetized with ketamine and xylazine, and restrained in plastic holders, then treated with laser light from an argon pumped dye laser at 700 nm for a total fluence of 135 J/cm² at a fluence rate of 75 mW/cm². The mice (6 mice/group in two groups) were checked daily, the tumors were measured using two orthogonal measurements L and W (perpendicular to L) and the volumes were calculated using the formula $V = L \times W^2 / 2$ and recorded. Mice were considered cured if there was no palpable tumor at 90 days post-PDT treatment.

The *in vitro* results obtained from the fluorinated and non-fluorinated photosensitizers at a dose of 0.25 µmL/kg are summarized in FIGURE 3, which shows comparative *in vitro* photosensitizing efficacy of fluorinated (9, 12) and non-fluorinated (8, 11) purpurinimide-based photosensitizers in RIF tumor cells, where the control shows photosensitizers only and no light. The *in vitro* results showed similar PDT efficacy of all purpurinimides. Compared to the *in vitro* results that showed similar PDT efficacy of all purpurinimides, the *in vivo* results were quite different. The *in vivo* results obtained from the fluorinated and non-fluorinated photosensitizers at a dose of 0.4 µmL/kg are summarized in FIGURE 4. FIGURE 4 compares the *in vivo* photosensitizing efficacy of fluorinated (9,12) and the corresponding non-fluorinated (8,11) purpurinimide-based photosensitizers in C3H/HeJ mice (6 mice/group). The mice were treated with light (706 nm, 135 J/cm²) at 24 hours post-injection. In the control group, six mice were exposed to light without injecting any photosensitizer. Among the fluorinated derivatives, purpurinimide 9 containing 3-O-(1'-butyloxy)ethyl and N-[3,5-bis-(trifluoromethyl)benzyl] substituents showed enhanced PDT efficacy (100% tumor response at day 90, *i.e.*, in each group 6/6 mice were tumor free) than the related isomer 12 [purpurin-18-N-butyl-3-O-{1'- 3",5"- bis-(trifluoromethyl)-benzyl ether} imide] producing 66% tumor response on day 90. Under similar treatment conditions, the non-fluorinated analogs 8 and 11 were found to be significantly less effective.

EXAMPLE 6

Determination of Tumor vs. Skin Uptake of Chlorin-related Compounds

For a compound to be biologically active under the PDT treatment conditions with reduced skin phototoxicity (a major drawback associated with most of the porphyrin-based compounds), it is necessary that it show a significantly higher accumulation in tumor than skin. Therefore, the uptake of the newly synthesized purpurinimide analogs **8**, **9**, **11** and **12** in tumor vs. skin was determined by *in vivo* reflectance spectroscopy (Zheng, G.; Potter, W. R.; Camacho, S. H., Missert, J. R., Wang, G., Bellnier, D. A., Henderson, B. W., Rodgers, M. A. J., Dougherty, T. J., and Pandey, R. K., *J. Med. Chem.* **44**: 1540-1559 (2001)). For these experiments, mice were first anesthetized using intraperitoneal administration of ketamine xylazine. The optical power as a function of wavelength was recorded before the i.v. injection of the photosensitizer. The drug was then injected and the spectrum was again recorded. The *in vivo* drug absorption spectrum is best displayed by determining the log of the ratio of the post-injection spectrum to the pre-injection spectrum. In a typical experiment, for measuring tumor uptake, the photosensitizers were injected into mice implanted with RIF tumors at a dose of 5.0 $\mu\text{mol/kg}$. All photosensitizers produced high tumor uptake and showed a considerable difference between tumor and skin levels 24 hour after injection, indicating enhanced tissue selectivity. The results for reflectance spectroscopy are shown for purpurinimide analogs **8**, **9**, **11** and **12** in FIGURES 5A, 6A, 7A and 8A, respectively, and the results showing the concentration of the compounds in RIF tumor versus C3H skin as a function of time are shown for purpurinimide analogs **8**, **9**, **11** and **12** in FIGURES 5B, 6B, 7B and 8B, respectively.

EXAMPLE 7

In vivo photosensitizing activity of Bacteriochlorin-related Compounds

The *in vivo* photosensitizing efficacy of bacteriochlorins **16** and **18** was determined in C3H mice (5 mice/group) at variable concentration up to 1.0 $\mu\text{mol/Kg}$. The experimental conditions used are those as described above

in EXAMPLE 5 for the purpurinimide analogs. The results obtained for bacteriochlorins **16** and **18** are shown in FIGURE 9 and FIGURE 10, respectively. Compared to compound **18**, bacteriochlorin **16** showed improved PDT efficacy. For example, bacteriochlorin **16** at a dose of 1.0 $\mu\text{mol/Kg}$ produced 80% tumor response (4/5 mice were tumor free at day 90), whereas at a same dose bacteriochlorin **18** produced 60% tumor response (3/5 tumor free at day 90). At a lower dose (0.4 $\mu\text{mol/Kg}$) bacteriochlorin **16** certainly showed improved photosensitizing efficacy (75% tumor cure at day 25 and 40% tumor cure on day 90) compared to **16** (day 15, 100% tumor cure and at day 25, no tumor response).

EXAMPLE 8

Tumor vs. muscle uptake of Bacteriochlorin-related compounds

The tumor vs. muscle uptake of bacteriochlorins **16** and **18** was determined in C3H mice by following the experimental conditions described for purpurinimide analogs. Both bacteriochlorins produced a considerably higher tumor uptake than the surrounding muscle. The results for reflectance spectroscopy are shown for of bacteriochlorins **16** and **18** in FIGURES 11A and 12A, respectively, and the results showing the concentration of the compounds in RIF tumor versus C3H skin as a function of time are shown for bacteriochlorins **16** and **18** in FIGURES 11B and 12B, respectively.

EXAMPLE 9

PDT Targeted at Helicobacter pylori

This EXAMPLE describes the clinical application of targeted photosensitizers to the treatment of an infection using PDT.

Helicobacter pylori is reportedly associated with tumors of the stomach in mice and as a putative agent of ulcerative pathology in humans. In this EXAMPLE, a capsular or pill-shaped and sized light source is administered orally to a patient, so that it passes into the stomach of the patient, where it administers light. Alternatively, an optical fiber may be passed into the stomach via the nasopharynx to administer light to the treatment site. In order to implement targeted PDT for treating ulcers in humans, an antibody that is targeted against a

suitable *Helicobacter pylori* antigen is conjugated to a compound provided herein and formulated into a pharmaceutical preparation that releases the conjugated compound to a gastric mucus/epithelial layer where the bacterium is found. The compound is ingested at a time when the stomach and duodenum is

5 substantially empty in order to promote binding of the compound to bacterium . Any unbound compound is diluted by gastric juice and carried distally by peristalsis to be eliminated from the body in fecal matter. Light sources suitable for intraluminal passage are disclosed in any one of U.S. Patent Nos.: 5,766,234; 5,782,896; 5,800,478; and 5,827,186, and pill or capsule shaped light sources

10 are disclosed in U.S. Patent No. 6,273,904, the disclosure of each being specifically hereby incorporated herein in its entirety. The light source is preferably energized just prior to its ingestion or remotely after ingestion, when in the stomach or in a desired intraluminal passage. If necessary, multiple light sources are ingested to insure that adequate photoactivation of the localized

15 compound occurs sufficient to kill the bacterium. The light source(s) may be deactivated after passage beyond the duodenum to avoid unwanted distal photoactivation. In this manner, a photosensitizing agent comprising the conjugated compound is activated topically without the need for a procedure such as endoscopy with fiberoptic gastric illumination in order to provide the

20 activating light. Since the compound is targeted, nonspecific uptake by normal tissue and other normal compositions of the body is minimized in order to prevent injury to normal gastric tissue and problems with the gastric system.

In this exemplary treatment, the following protocol may be utilized:

Step 1 Patient is NPO for six hours to insure that the stomach is empty.

25 Step 2 The targeted compound is ingested.

Step 3 One hour elapses to allow for bacterial binding and distal passage of unbound compound. The optimal period can be longer or shorter and is readily determined by measuring the clinical response; for example, response can be determined endoscopically by observation and biopsy.

30 Step 4 One or more light sources are ingested sequentially and activated in the stomach. The length of time that light is administered by these sources and the number of sources that are ingested will be determined clinically in a

light dose escalation study. The churning action of the stomach serves to translocate the light source(s) so that the light is distributed more evenly prior to passage of the source(s) into the duodenum. Since each light source is small (the size of a pill or tablet), it passes easily out through the GI system via

5 peristalsis.

Step 5 The light sources are deactivated after distal passage beyond the gastroduodenal area and excreted in fecal matter.

Note that it is also contemplated that an external light source located over the gastric area can be used to transcutaneously administer light to the treatment

10 site. The use of an external light source requires that the targeted compound and the light source absorb and emit in the near infrared to infrared range, respectively, so that the light will efficiently penetrate the patient's skin and reach the treatment site.

EXAMPLE 10

15 ***PDT for Targeting Pulmonary Tuberculosis***

An target compound provided herein is formulated to bind with great affinity to *Mycobacterium tuberculosis* in a selective and specific manner. Preferably, the targeted compound is formulated as an aerosol, which can be easily inhaled, enabling distribution into all lung segments. Steam is then inhaled

20 to solubilize any unbound targeted compound and facilitate its removal from the lung by exhalation. Alternatively, the targeted compound is formulated as an injectable compound and administered intravenously. Either way, the bound targeted compound is photoactivated by an external light source disposed on the chest and/or back.

25 Step 1 The targeted compound is inhaled or injected.

Step 2 Time is allowed to elapse to allow binding of the targeted compound with the *Mycobacterium tuberculosis*, followed by steam inhalation to remove any unbound compound (if inhaled). The time required to ensure a therapeutically effective dose of bound targeted compound may be routinely

30 determined clinically using standard clinical practices and procedures.

Step 3 The light source is disposed adjacent to the thorax and activated for a sufficient time to ensure that therapeutic irradiation has occurred, which may be routinely determined clinically using conventional clinical practices and procedures. The fluence rate and total fluence dose may be determined as
 5 noted above.

Note that alternatively, an internal light source disposed within the thoracic area can be used to administer the light. The use of an external light source requires that the targeted compound and the light source respectively absorb and emit light in the near infrared to infrared range to ensure efficient skin
 10 penetration of the light.

EXAMPLE 11

PDT for Targeting Otitis Media

A photosensitizer conjugate is formulated which binds with great affinity to *Streptococcus pneumoniae* and *Hemophilus influenzae* in a selective manner.

15 The targeted conjugate is formulated into an injectable, which can be administered intravenously or instilled topically into the middle ear via a previously placed tympanostomy tube. The drug is activated using light emitted by a small light source about the size, shape, and weight of a hearing aid, which is disposed behind the ear and aimed at the middle ear, so that the light passes
 20 into the middle ear transcutaneously.

Step 1 The targeted compound fluid formulation is instilled into the middle ear.

Step 2 Sufficient time is allowed to elapse to allow binding of the targeted compound with the disease organisms, and then, any excess fluid is drained
 25 away by gravity or actively aspirated using a needle and syringe.

Step 3 The light source is positioned behind the ear and activated. The light source need not be very intense since the middle ear cavity is small.

Since modifications will be apparent to those of skill in this art, it is
 30 intended that this invention be limited only by the scope of the appended claims.